THE ROLE OF NATURAL SELECTION AND ADAPTATION VERSUS PHENOTYPIC PLASTICITY IN THE INVASIVE SUCCESS OF *HIERACIUM LEPIDULUM* IN NEW ZEALAND.

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by Seija Anna Parkkali

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Table of Contents

List of Figures

List of Tables

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Abstract

Hieracium lepidulum is an invasive weed in New Zealand. It colonises a wide range of habitats including pine plantations, scrubland, native *Nothofagus* forest, and mid-altitude to alpine tussock grassland, where it is competing with indigenous species. Understanding the breeding systems and population genetic structure of *H. lepidulum* is important for biocontrol, and aids in the understanding of evolutionary colonisation processes. *H. lepidulum* is a triploid, diplosporous, obligate apomict. This type of reproduction through clonal seed does not involve meiosis or fertilisation, and theoretically populations should contain very low levels of genetic variation, the only source being somatic mutation. Common garden experiments and microsatellite markers were used to determine the population genetic structure of *H. lepidulum* populations in the Craigieburn Range, Canterbury. Both experiments revealed that populations, sampled from three replicate altitudes within three geographically-separated locations, contained no genetic variation; individuals all possessed the same microsatellite genotype. These results strongly suggest that the Craigieburn Range *H. lepidulum* individuals reproduce solely by apomixis and populations belong to the same clonal lineage. Populations were also examined for their response to two abiotic environmental 'stresses', drought and shade. *H. lepidulum* populations' exhibited high drought tolerance, yet appeared to be shade-intolerant. Low levels of reproduction in light-limiting habitats will prevent the invasion of *H. lepidulum* into closed-canopy forest habitats. *H. lepidulum* appears to have overcome the reduction in fitness associated with apomictic reproduction by phenotypic plasticity, fixed heterozygosity and polyploidy – all associated with increased vigour, fitness, and the ability to occupy broader ecological niches. This study's results are hopeful for the development of biocontrol programs involving genotype-specific pathogens but suggest that grazing management may not succeed. The data will be useful for future comparisons of genetic structure during the course of *H. lepidulum* invasions and will contribute to the management of this invasive weed.

Chapter 1

1. Introduction

1.1 The biology of exotic plant invasions

Biological invasions arise from the successful proliferation and establishment of a species in a non-native region (Muller-Scharer *et al*. 2004, Facon *et al*. 2006). Plant invasions are now a major focus of research as they are becoming progressively more frequent owing to global trade and transport (Muller-Scharer *et al*. 2004, Bossdorf *et al*. 2005, Lockwood *et al*. 2005, Richards *et al*. 2006). The results of exotic invasions are detrimental; they not only jeopardise biodiversity, including displacement of native vegetation, disrupt community organisation and ecosystem function but are also damaging to agriculture, public health and fisheries (D'Antonio and Vituosek 1992, Sakai *et al*. 2001, Keane and Crawley 2002, Lee 2002, Hierro *et al*. 2005, Ortega and Pearson 2005, Facon *et al*. 2006).

1.1.1 The process of invasion

Plant invasions take place in a series of stages. Invasion begins with the introduction of propagules into the new region, followed by establishment, population growth, and then spread into new locations (Dietz *et al*. 1999, Mack *et al*. 2000, Sakai *et al*. 2001, Hall *et al*. 2006).

1.1.2 Establishment

The likelihood of successful establishment after introduction to the new region can be determined by habitat resistance to invasion and propagule pressure (Muller-Scharer *et al*. 2004, Facon *et al*. 2006, Mitchell *et al*. 2006).

Environmental resistance to establishment can be an important factor in the success of the exotic invader. Habitat resistance is correlated with the availability of safe sites for establishment which can be determined by; abiotic resource availability (e.g., light, moisture, and nutrients), biotic interactions (e.g., competition with resident plants, predation, herbivory, and disease), and disturbance events (Richardson *et al*. 2000, Levine *et al*. 2004, Parker and Hay 2005, Facon *et al*. 2006).

Propagule pressure may be the most critical factor in the establishment success of exotic species in a new range (Sakai *et al*. 2001, Lockwood *et al*. 2005). Propagule pressure is a measure of both the number of individuals in each release event (propagule size), and the number of separate release events (propagule number) (Lockwood *et al*. 2005). An invading population has a greater chance of surviving, establishing and spreading in a heterogeneous environment if the foundering pool is large. A large founder population is more likely to possess greater genetic variability than a small colonising population and therefore has a greater chance of comprising individuals that are genetically suitable in the new range to occupy the available safe sites (Lee 2002, Ahlroth *et al*. 2003, Muller-Scharer *et al*. 2004). An invading population is also more likely to come across appropriate microsites for establishment if there are multiple introductions (Mack *et al*. 2000, Kolar and Lodge 2001,

Ahlroth *et al*. 2003, Lockwood *et al.* 2005). These multiple introductions may also provide additional genetic variation to the invading population (Sakai *et al*. 2001).

1.1.3 Population growth

The establishment of a non-native species into a new range does not ensure invasion success; the invader must be able to sustain rapid expansion in both the size and number of populations (Mack *et al*. 2000). Plant invasions frequently have a lag time between the initial establishment and the commencement of rapid population growth (Sakai *et al*. 2001, Facon *et al.* 2006). This lag time has been suggested to involve the rapid evolution of the invading species. This process may include; the adaptation of the invading species to the new range, intra- or interspecific hybridisation, the purging of genetic load responsible for inbreeding depression, and the evolution of increased vigour and invasiveness (Ellstrand and Schierenbeck 2000, Sakai *et al*. 2001, Lee 2002, Muller-Scharer *et al*. 2004, Bossdorf *et al*. 2005, Hierro *et al*. 2005, Facon *et al*. 2006). Adequate additive genetic variance (the variance of particular phenotypic traits that responds to natural selection) is vital for the adaptation and evolution of the invading species in response to environmental variation (Lee 2002, Muller-Scharer *et al.* 2004). The time lag allows time for this additive genetic variance to accumulate and then the subsequent rapid evolution of the invading species (Sakai *et al*. 2001, Lee 2002, Lockwood *et al*. 2005, Facon *et al*. 2006).

1.1.4 Range expansion

The last step in the process of invasion involves the range expansion of the exotic species (Mack *et al.* 2000, Sakai *et al*. 2001). The rate of spread of the invading population is

associated with both the survival and reproductive rates of the species in the new range and its ability to disperse adequately (Sakai *et al*. 2001).

1.2 Theories for mechanisms of invasion

When a non-native species invades a new range it encounters abiotic and biotic factors which may be different from those in its native range, including novel environmental gradients and a variety of habitats. The exotic species must be able to overcome these differences to successfully invade the new range. There are various theories behind the mechanisms for success of invasive species in their new habitats, including selection and adaptation, phenotypic plasticity, 'general-purpose genotypes' and the 'frozen-niche hypothesis'.

1.2.1 Natural selection and adaptation

Charles Darwin was the first to propose the idea of natural selection, the force which drives the adaptive evolution of populations. His theory was that populations produce more offspring than the environment can sustain, which leads to a 'fight for survival'. This competition favours individuals which have traits associated with strong competitive ability and are most suited to the environment; these individuals will prosper and reproduce. Over generations, the strong-competitive traits are selected for and the weak traits eventually vanish. In this way natural selection can lead to the adaptation and evolution of populations.

The adaptation and evolution of a species via natural selection requires the presence of genetic variation among individuals in a population upon which natural selection can act (Barrett 1982, Sakai *et al*. 2001, Lee 2002, Muller-Scharer *et al*. 2004). An exotic species

may evolve during the initial establishment and also during range expansion (Sakai *et al*. 2001, Lee 2002). Throughout the invasion process exotic species will encounter various selection pressures in the new environment such as different environmental gradients (e.g. temperature, photoperiod, and climate), or resident species (e.g. as competitors, predators, or prey). Rapid evolution may occur via selection and adaptation in response to these selection pressures giving the invading species the ability to spread in heterogeneous landscapes (Sakai *et al*. 2001, Lee 2002, Facon *et al*. 2006).

1.2.2 Phenotypic plasticity

Phenotypic plasticity is defined as the ability of a single genotype to develop multiple phenotypic states depending on the environmental conditions (Barrett 1982, Miner *et al*. 2005). Plastic responses to the environment can include changes in morphology, physiology, behaviour, life history, growth, and demography (Miner *et al*. 2005). Plastic responses can occur within the lifespan of an individual or across generations (Miner *et al.* 2005). Phenotypic plasticity is thought to be important in the process of invasion as it allows the colonising species to succeed in a variety of habitats and environments (Sakai *et al.* 2001, Bossdorf *et al*. 2005). Phenotypic plasticity would bestow a fitness advantage on an invading species which suffered from a lack of genetic variation, and thus preventing adaptation via natural selection (Bossdorf *et al*. 2005).

Phenotypic plasticity is a trait which can be under selection itself. Phenotypic plasticity is essentially controlled at the genetic level; a single genotype can express multiple phenotypes in variable environments. Studies have found the amount and pattern of genetic variation that

is expressed can be environment dependent (i.e. genetic variation for plasticity among habitats) and that there is also genetic variation for plasticity within populations (Pigliucci *et al*. 1995, Volis *et al*. 2002, Pigliucci 2005 and refs. within, Kovnat 2007, Bell and Galloway 2008). As variation is a necessity for natural selection, the plastic expression of a genotype could therefore evolve due to variation in the loci expressing the plastic phenotypes, resulting in adaptive phenotypic plasticity occurring in natural populations (Pigliucci *et al*. 1995, Pigliucci 2005, Bell and Galloway 2008).

1.2.3 General-purpose genotype

The hypothesis of a 'general-purpose genotype' is similar to that of phenotypic plasticity but is used in association with clonal species. Clonal lineages reproduce asexually and members of a lineage are expected to be genetically identical, apart from rare differences through somatic mutation (Fox *et al.* 1996, Storchova *et al*. 2002). This hypothesis relies on the assumption that clones are generalists (often due to polyploidy) and have the ability to occupy all habitats across a heterogeneous environment, similar to phenotypic plasticity (Fox *et al*. 1996). These generalist clones will also produce widely-adapted offspring enhancing their success of invasion, whereas widely-adapted sexuals will generate fewer broadly-adapted offspring due to recombination (Fox *et al*. 1996).

1.2.4 Frozen-niche hypothesis

The frozen-niche hypothesis is the reverse of the general-purpose genotype hypothesis in that it involves specialist clones as opposed to generalists. Clonal genotypes and their adaptations are 'frozen' for a specific niche and the most successful specialists have the least overlap of

niche space with other clones (Fox *et al*. 1996). In this scenario clonal diversity is due to the multiple origins of clonal lineages and this diversity is preserved by the presence of a variety of niches (Fox *et al*. 1996). Exotics utilising this mechanism for invasion would be successful as they would be able to colonise a variety of habitats in the new environment.

1.3 Summary

One of the big questions in evolutionary biology at the moment is determining what mechanisms are most important in the invasion process. Understanding whether genetic variation, via selection and adaptation, or phenotypic plasticity is more important in the success of an invading species is crucial to aid in the development of biocontrol mechanisms. The aim of this study is to determine whether natural selection and adaptation, or phenotypic plasticity is more important in the success of the invasive weed *Hieracium lepidulum* in New Zealand. Understanding the evolutionary colonisation processes of this weed is essential in order to control and eventually eradicate this destructive invader.

1.4 The study species, *Hieracium lepidulum*

Figure 1.1: *Hieracium lepidulum*.

1.4.1 The biology of *Hieracium lepidulum* and its introduction to New Zealand

Hieracium lepidulum (Figure 1.1), family Asteraceae, is a seriously invasive weed in New Zealand. *H. lepidulum* is native to Northern and Central Europe where it resides in a variety of habitats, but is predominantly a forest herb (Wiser and Allen 2000, Chapman *et al.* 2004). It is thought to have been introduced to New Zealand in the late 1800s via contamination of imported European grass seed (Espie 1994, Chapman *et al.* 2004). Many long-distance introductions of exotic species to new regions are caused, either directly or indirectly, by human activities (Sakai *et al.* 2001). The first recorded occurrence of *H. lepidulum* in New Zealand is in the creek-margin habitat in the Craigieburn Range, Canterbury, in 1941. In the next two decades it was recorded at Lake Wanaka, Central Otago, in 1950 and in the

Nelson/Marlborough region in 1964. These three areas are thought to have acted as centres of spread for *H. lepidulum* in New Zealand (Miller 2005). After the initial recordings there was a 40-year lag phase, this was followed by rapid exponential growth during the 1980s and then in the early 1990s there was a decline in the rate of spread of *H. lepidulum* (Miller 2005).

1.4.2 Abundance of *Hieracium lepidulum* and its threat to indigenous species

Although exotic plant invasions are a major problem worldwide, New Zealand is seriously suffering with established exotic species making up approximately half of the vascular plant flora (Owen 1998). Non-native plant species are deemed a threat to the survival of 59 % of New Zealand's indigenous flora (Dopson *et al*. 1999). Over the last 50 years the frequency and abundance of *H. lepidulum* has been progressively increasing throughout the South Island of New Zealand. *Hieracium* (hawkweeds) are now the dominant vegetation cover for over 500 000 hectares in the South Island (Duncan *et al.* 1997). In the Rob Roy catchment in Central Otago, parts of mid-Canterbury, and the Borland Mire in Southland, *H. lepidulum* has formed dense meadows with close to 100 % cover (Figure 1.2) (Chapman *et al.* 2004).

H. lepidulum has the ability to invade a wide range of habitats, including pine plantations, scrubland, native *Nothofagus* forest, and mid-altitude to alpine tussock grassland (from 750 m to 1700 m in altitude) (Treskonova 1991, Rose *et al*. 1995, Duncan *et al*. 1997, Chapman *et al.* 2004). As this species is capable of invading into, and dominating, such a wide range of habitats it poses a serious threat to indigenous plant communities (Connor 1992, Wiser *et al.* 1998, Chapman *et al*. 2004).

Figure 1.2: A dense meadow of *Hieracium lepidulum.*

1.4.3 Mode of reproduction

The genus *Hieracium* is well known for being taxonomically complex because of its variety of breeding systems and ploidy levels (Storchova *et al*. 2002). *H. lepidulum* reproduces through the production of clonal seed; specifically it is a diplosporous, obligate apomict (Chapman *et al*. 2004). Apomictic processes occur in the ovule. In diplospory the embryo arises directly from an egg cell in an unreduced embryo sac (Asker and Jerling 1992, Kultunow *et al*. 1995). This type of asexual reproduction through seed is a breeding system which does not involve meiosis or fertilisation, and is essentially a complete transmission of the entire maternal genotype to the offspring (Asker and Jerling 1992, Kultunow *et al*. 1995, Chapman *et al*. 2004). Apomixis fixes a particular genotype, and apomicts are therefore described as clonal lineages (Asker and Jerling 1992, Kultunow *et al*. 1995, Storchova *et al*. 2002, Chapman *et al*. 2004). This mechanism of reproduction results in a colossal number of asexual clones, often with different clones dominating in different populations; theoretically

the populations should harbour very low levels of genetic variation (Bayer *et al.* 1990, Storchova *et al*. 2002, Chapman *et al*. 2004). Low levels of genetic variation are typically associated with evolutionary dead ends, because natural selection has very little variation on which to act (Fox *et al*. 1996). However apomicts are generally polyploid and *H. lepidulum* is triploid (Bierzychudek 1989, Asker and Jerling 1992, Chapman *et al*. 2004). This means it has three copies of each chromosome, and so has the potential for more variation than in a vegetatively-reproducing diploid. Moreover, some variation can be created in clonal triploids through simple mutation, autosegregation, hybridisation, and somatic recombination (Storchova *et al*. 2002, Chapman *et al*. 2004). There have been no recordings of sexual diploids in *H. lepidulum*, so intraspecific hybridisation as a means of providing genetic variation is very unlikely (Chapman *et al*. 2004). Studies examining morphological and genetic analyses have exposed clonal lineages which contain an unexpected amount of diversity, which questions whether obligately apomictic species really do exist (Bayer *et al*. 1990, Fox *et al*. 1996, Chapman *et al*. 2004). Apomictic reproduction is associated with phenotypic plasticity, fixed heterozygosity and polyploidy; these traits are linked with increased vigour, fitness, and the ability to occupy broader ecological niches (Asker and Jerling 1992, Soltis and Soltis 2000, Joly and Bruneau 2004, Comai 2005, Andersen *et al*. 2006). The invasive success of *Hieracium lepidulum* could possibly result from phenotypic plasticity, fixed heterozygosity and polyploidy, which could overcome the reduction in fitness typically associated with apomictic reproduction. Understanding the population genetic structure of *H. lepidulum* will aid in the development of biocontrol programmes to manage this invasive weed. The population genetics will also help us understand the

evolutionary colonisation processes associated with the invasion of *H. lepidulum* in New Zealand.

1.5 The study site, Craigieburn Range

Figure 1.3: The Craigieburn Range.

The site I have chosen for my research is the Craigieburn Range (Figure 1.3), located in the mid-Canterbury region of the Southern Alps (43°12' S, 171°40' E). This is an ideal study site as it is where *Hieracium lepidulum* was first recorded in New Zealand in 1941, and has advanced from a rare occurrence in 1956 to a seriously invasive weed (Scott 1993, Chapman *et al*. 2004). The majority of the Craigieburn Range is managed as Craigieburn Forest Park for conservation, education, and recreation. The range boasts a mountainous landscape, with altitudes extending from 700 m to 2000 m. The lower elevations are occupied by lowland short-tussock grasslands; mountain beech forest (*Nothofagus solandri* var. *cliffortiodes*) dominates from ~800 m to 1300 m, where it subsides to subalpine and alpine grassland and scrubland (Leamy and Fieldes 1976, Chapman *et al*. 2004). The predominant soils of the region are high-country yellow-brown earths (Leamy and Fieldes 1976). The mean annual rainfall of the Craigieburn Range is 1446 mm (Environment Canterbury, pers. comm. 2008) and the mean annual temperature is 7.4 °C (Arthur's Pass Weather Station, www.softrock.co.nz).

This study involved three geographically-separate catchment locations within Craigieburn Forest Park: Mount Cheeseman Skifield, Broken River Skifield, and Craigieburn Skifield basins. Throughout the thesis these three locations will be referred to as; 'Cheeseman', 'Broken River', and 'Craigieburn'. Within each location, populations were sampled from three different altitudes, which define three different habitats (Figure 1.4):

- Lowland close to the road, 750 m in altitude.
- Mid-forest, 1100 m.
- Above the tree line in the alpine tussock grassland, 1450 m.

This gave three replicates of each habitat, where each replicate is from one of the three geographically-separate locations (Figure 1.5). Throughout the thesis these altitudes/habitats will be referred to as; 'lowland', 'forest', and 'alpine'.

1.6 The aim and structure of this thesis

1.6.1 Overall aim

The overall aim of this thesis is to determine whether genetic variation via natural selection and adaptation, or phenotypic plasticity is more important in the success of the invasive weed *Hieracium lepidulum* in New Zealand.

To understand which mechanism (natural selection and adaptation, or phenotypic plasticity) is more important in the process of invasion for *H. lepidulum* I have undertaken various glasshouse experiments and genetic analyses. Determining the amount of genetic variation present within and between populations, the population genetics, will help in the

understanding of the evolutionary colonisation processes of *H. lepidulum* and aid in the development of biocontrol programmes for this invasive weed.

1.6.2 Thesis outline

The remainder of the thesis will be split into four major sections. The following three sections will be dedicated to the three experimental components of the research; the common garden experiment, the degree of phenotypic plasticity in response to environmental stress and the genetic analysis. The final section will then assemble all the results in a general discussion of the research and its significance to the management of this invasive weed, *Hieracium lepidulum*.

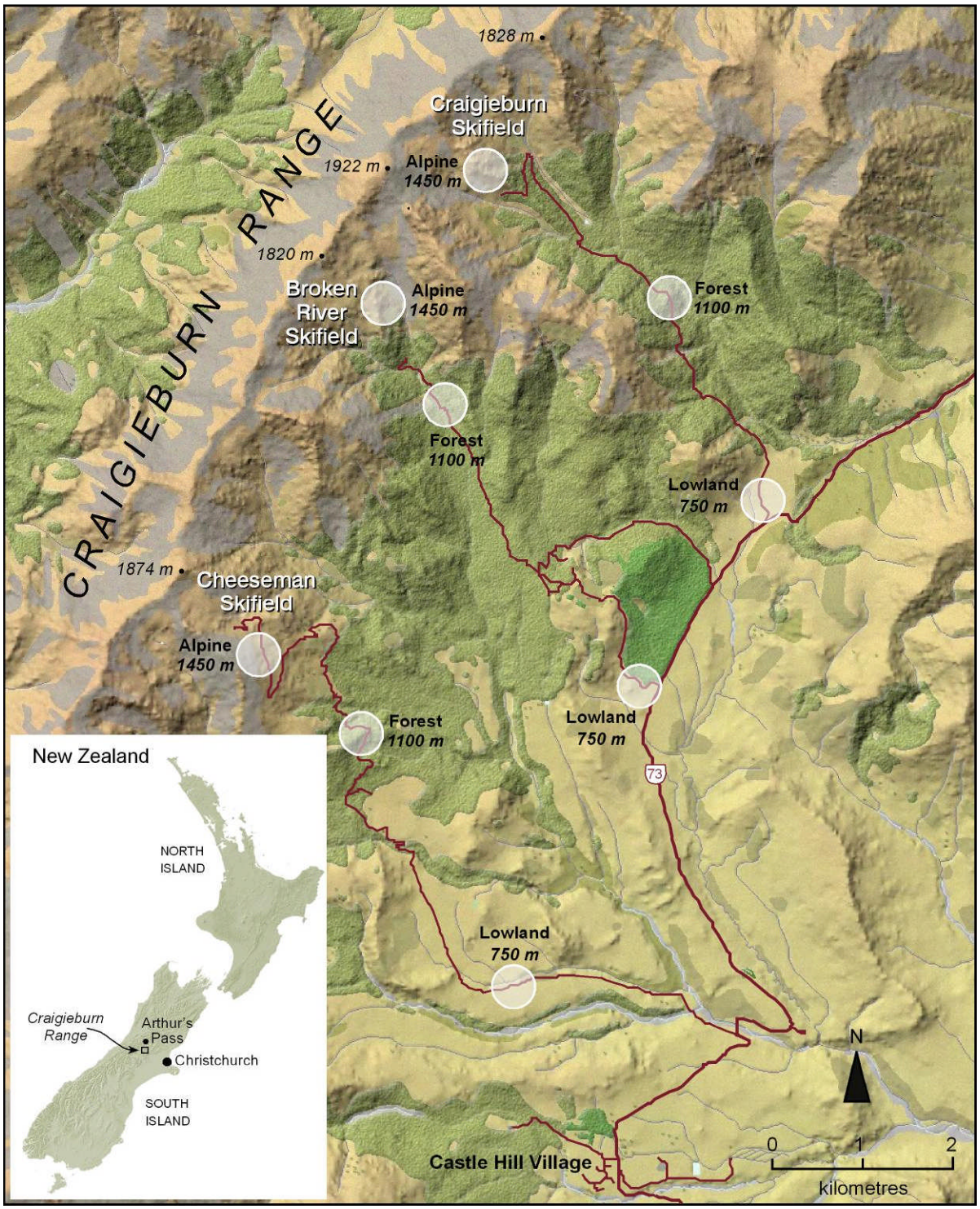


Figure 1.4: Sites map; illustrating the three geographically-separated catchment locations, and within each location the three different altitudes the populations were sampled from (white circles). (Sandra Parkkali, Department of Conservation).

Figure 1.5: Examples of the three types of habitat used in sampling: alpine (top), forest (middle), lowland (bottom).

Chapter 2

2. What is more important in the invasive success of *Hieracium lepidulum*; adaptation via natural selection acting on genetic variation, or phenotypic plasticity?

2.1 Introduction

Hieracium lepidulum is a major threat to the indigenous flora of New Zealand. The invasion success of *H. lepidulum* is due in part to its great ability in colonising a wide range of habitats, including; pine plantations, scrubland, native *Nothofagus* forest, and mid-altitude to alpine tussock grassland (from 750 m to 1700 m in altitude) (Treskonova 1991, Rose *et al*. 1995, Duncan *et al*. 1997, Chapman *et al*. 2004). The two main mechanisms which would allow a plant to be able to survive in a variety of habitats are; natural selection acting on genetic variation leading to adaptation, or phenotypic plasticity.

Natural selection acts on genetic variation among individuals in a population; the most suitable genotypes will survive and reproduce more than others. Over generations this natural selection leads to the adaptation and evolution of the population. When a population faces a variable environmental gradient, natural selection will favour individuals which have traits that are most suited to the environment. For example, populations residing in an alpine habitat will be confronted with much different environmental selection pressures than those in low-lying grassland. If natural selection is responsible for the adaptation of populations of *H. lepidulum* to the variety of habitats it occupies, then these populations must be genetically

variable. The more different the habitats are from each other, the more genetically diverse the populations should be. Natural selection and adaptation can result in plant populations from different habitats exhibiting very different phenotypic characteristics.

Phenotypic plasticity is the ability of a single genotype to develop multiple phenotypic states depending on the environmental conditions; it is a phenomenon which does not require genetic variation to produce multiple phenotypes due to the 'general-purpose genotype' (Barrett 1982, Miner *et al*. 2005). Plastic responses to the environment can include; changes in morphology, physiology, behaviour, life history, growth, and demography (Miner *et al*. 2005). Phenotypic plasticity is common in polyploid, clonal organisms; it provides populations with the ability to prosper in heterogeneous environments (Bierzychudek 1989, Asker and Jerling 1992). Plastic responses can occur within the lifespan of an individual or across generations (Miner *et al*. 2005). Plasticity is thought to be very important in the process of invasion as it allows the exotic to respond within a single generation to their new environment to escape a decrease in fitness (Sakai *et al*. 2001, Bossdorf *et al*. 2005). Phenotypic plasticity would bestow a fitness advantage for an invading species which suffered from a lack of genetic variation preventing adaptation via natural selection (Bossdorf *et al*. 2005).

2.1.1 Phenotypic differences among populations of *Hieracium lepidulum* in the field

A recent study (Miller 2005) discovered that there were significant phenotypic differences among populations of *Hieracium lepidulum* which were occupying different, but geographically close, habitats in the Craigieburn and Broken River catchments. This study

looked at seven microhabitats which were commonly invaded by *H. lepidulum*; forest creek, forest edge, forest interior, forest canopy gap, alpine creek, alpine tussock, and alpine scrub. Plant performance measures were recorded for each individual in the different populations, which allowed the comparison of performance among habitats. Plant performance was assessed by three measures:

1) Plant size (by measuring length of longest leaf, as it is relative to plant size).

2) Reproductive output (number of flowering stems, buds, inflorescences and seed heads).

3) Mortality (discarded as rates were too low over study period).

Miller's results found that plant performance differed among the seven habitats. Overall the alpine habitats had a higher level of plant performance than the forest habitats. Leaf lengths were greater in the alpine habitat, therefore plants were larger in size, and plants in the alpine habitats were also more fecund than in the forest habitats. From this work, we now know that *H. lepidulum* populations which occupy different habitats show marked variations in their phenotypes. There are two main explanations for this phenomenon; either the alpine populations are genetically different to the others, or this species is plastic. A combination of adaptation and phenotypic plasticity is also possible.

2.1.2 Objective one

The first objective of my research is to determine whether the populations of *Hieracium lepidulum* which occupy different habitats, in close geographic proximity, show patterns of genetic variation among populations which are consistent with adaptation, or whether its successes and widespread distribution in the Craigieburn Range looks more likely to be the result of phenotypic plasticity.

2.2 Methods

2.2.1 Experimental background

A standard way to investigate whether phenotypic differences observed in the field are the result of phenotypic plasticity, or genetic variation via natural selection and adaptation, is the use of common garden experiments (Barrett 1982, Tellez and Moller 2006). This involves sampling populations from different habitats in the field and transferring them into strictly controlled, uniform conditions, a 'common garden'. Each individual in the common garden will experience as near identical growing conditions (e.g. light, water, nutrients, and temperature) as possible (Barrett 1982). If individuals from different environments maintain their morphological/physiological differences in a common garden, these differences must be genetic. However, if the different morphotypes are lost when grown in a common garden, these differences must be due to phenotypic plasticity.

2.2.2 Population locations and sampling

Populations of *H. lepidulum* were sampled from three geographically-separate catchment locations within Craigieburn Forest Park; Mount Cheeseman Skifield, Broken River Skifield, and Craigieburn Skifield basins. These three locations will be referred to as; 'Cheeseman', 'Broken River', and 'Craigieburn'. Within each location, populations were sampled from three different altitudes, which define three different habitats; **(1)** lowland close to the road, 750 m in altitude, **(2)** mid-forest, 1100 m, and **(3)** above the tree line in the alpine tussock grassland, 1450 m (refer to Figure 1.4 for sites map). These altitudes/habitats will be referred to as; 'lowland', 'forest', and 'alpine'. This gave three replicates of each habitat, where each

replicate is from one of the three geographically-separate locations. A total of nine populations of *H. lepidulum* were used for the common garden experiment:

- Broken River – lowland, forest and alpine
- Cheeseman – lowland, forest and alpine
- Craigieburn – lowland, forest and alpine

Populations of *H. lepidulum* were sampled from the Craigieburn Range in November 2007. From each of the nine populations I randomly sampled 34 individuals; each individual was at least 10 m away from the other to ensure no sampling from the same vegetative clone. This gave a total of 306 individuals for the common garden experiment. Each individual sampled was at an early growth stage, as a very small rosette, to reduce the variation in starting size.

2.2.3 Common garden setup

Two tray tables were set up in a glasshouse at the University of Canterbury, Christchurch. Each table was lined with black mesh to inhibit weeds and then filled with fine gravel to aid in drainage. The samples of *Hieracium lepidulum* collected from the field were planted in individual pots (10-cm diameter, 10-cm deep) with standard soil mix. The common garden was watered thoroughly twice a week until the gravel was saturated. A random-blocking technique was used to arrange the pots to minimise the clustering of populations purely by chance and also to control systematic factors (e.g. light and temperature) which may affect the experiment.

2.2.4 Phenotypic measurements

To determine which mechanism (phenotypic plasticity or genetic variation via selection and adaptation) is more important in the invasive success of *Hieracium lepidulum*, the plant performance of each individual was recorded over time. The plant performance measures monitored were (Figure 2.1):

- maximum leaf length
- number of flowering stems
- number of buds
- number of inflorescences
- number of days until first flower
- number of seed heads
- number of seeds per seed head

These particular performance measures were chosen because Miller (2005) had already demonstrated variation among these phenotypic traits in the field. The length of the longest leaf is commonly used as a relative measure of plant size (Wesselingh *et al*. 1997, Buckley *et al*. 2003). The other six performance traits give a measure of the reproductive output of each individual (Pigliucci *et al.* 1995, Miller 2005).

For analysis, the maximum value recorded for each individual over the entire study period was used for leaf length, number of flowering stems, buds and inflorescences. The sum of all seed heads produced throughout the study period for each individual was used for analysis. Fifteen seed heads were sampled from each individual and seeds counted to give a mean value for the number of seeds per seed head. Each performance indicator was measured on a

weekly basis for 32 weeks. Initial plant sizes were obtained by measuring rosette size to determine any variation in starting size that may affect the final growth performance results.

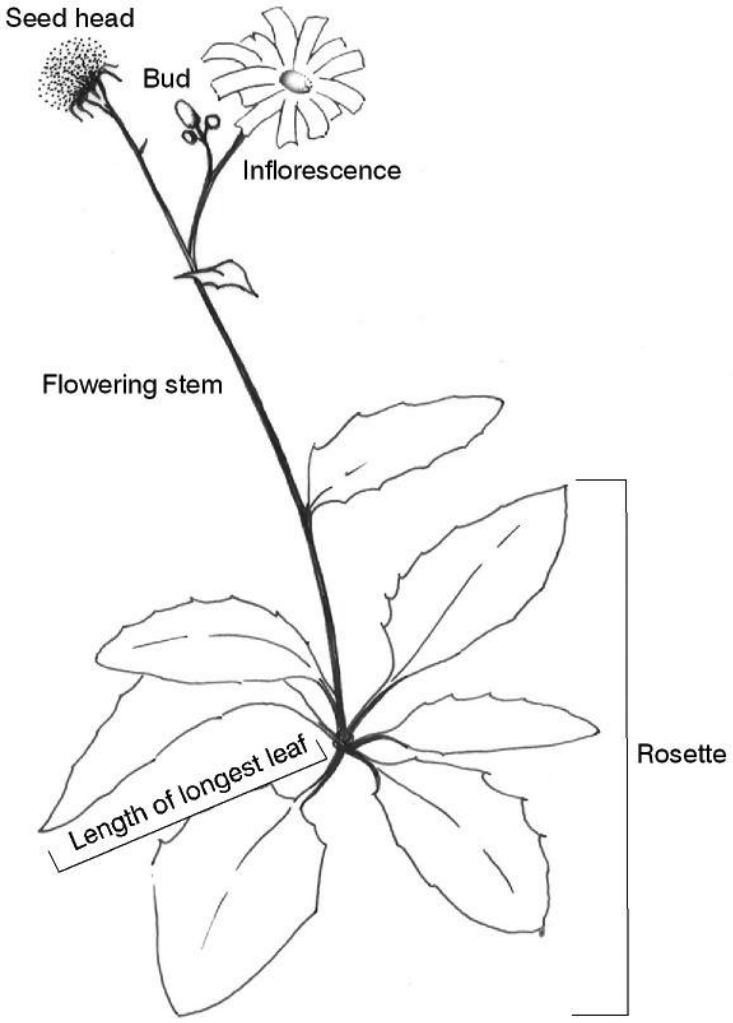


Figure 2.1: Diagram of *Hieracium lepidulum* illustrating the phenotypic traits measured.

2.2.5 Analysis

Tests for differences among populations, in terms of the performance measures in the common garden experiment, were made using analysis of variance (ANOVA). The two main factors were habitat and location, and their interaction. Habitat represents the three different habitats used in sampling; 'lowland', 'forest' and 'alpine'. Location represents the three geographically-separated locations ('Broken River', 'Cheeseman' and 'Craigieburn') in which the three habitats were replicated. The interaction between habitat and location (habitat*location) determines the variation among the nine populations for the performance traits. The null hypothesis for each factor and their interaction was that there would be no statistically significant difference among them for the performance measure.

Frequency distributions were determined for all performance traits. The statistical distributions were skewed for some of the count variables (number of buds, number of flowers, days until first flower, and total number of seed heads). This data required transformation, either $\log_e$ or square root, to satisfy the assumptions of the analysis (homogeneity of variance and normality). ANOVAs were then determined for all seven performance traits.

Multiple pairwise comparisons of the means by the Tukey's Honest Significant Differences (Tukey's HSD) method was used to determine which specific population means differ from which others, as ANOVA results only tell us that at least one of the means is significantly different from the others. The Tukey's HSD test applies a value of $\alpha = 0.05$ to the whole analysis (comparison of all the means) instead of for each single comparison. This sets the

family-wise error rate, reducing the α value for each specific comparison, thus avoiding the problem of a Type I error occurring. Initial size at planting did not vary among the populations, therefore it did not need to be included as a covariate in the analyses as it will not have any influence on the final performance outcome. All data analysis was done using R 2.6.0 (R Development Core Team 2007).

2.3 Results

Analysis of variance was carried out on each performance measure to determine if there were any significant differences present among habitats, locations and populations. A significant ANOVA result for habitat would suggest that there is genetic variability among habitats, across the three locations. A significant result for location would suggest that there is genetic variability among the locations, irrespective of habitat. If the interaction between habitat and location is significant it implies there is genetic variation among the nine populations. The null hypothesis being that there are no significant differences for the performance traits.

The ANOVA results indicated that there were no significant differences either among habitats or locations for any of the performance measures in the common garden (Table 2.1). This result suggests that there is no genetic variability among the habitats or among the locations. The habitat*location interactions were found to be not significant for all the performance measures except for the number of flowering stems. A non-significant habitat*location interaction implies there are no statistical differences among the population means for maximum leaf length, number of buds, inflorescences, days until first flower, total number of seed heads and seeds per seed head (Figure 2.2). Number of flowering stems has a

slightly significant (p = 0.0422) result which indicates that at least one of the population means differ from the others for this performance trait.

Performance measure	Habitat		Location		Habitat*Location	
	F value	p value	F value	p value	F value	p value
Maximum leaf length	1.8221	0.1636 ns	0.6990	0.4980 ns	1.1765	0.3213 ns
Number of flowering stems	0.2905	0.7481 ns	1.7986	0.1674 ns	2.5084	0.0422 *
Number of buds	1.3807	0.2531 ns	0.4126	0.6623 ns	0.3199	0.8645 ns
Number of inflorescences	2.1203	0.1219 ns	2.2148	0.1111 ns	0.3854	0.8190 ns
Days until first flower	0.3188	0.7273 ns	0.9648	0.3823 ns	0.4029	0.8065 ns
Total number of seed heads	0.7735	0.4626 ns	1.3784	0.2537 ns	0.7634	0.5498 ns
Seeds per seed head	0.0335	0.9671 ns	0.0359	0.9647 ns	0.0175	0.9994 ns

Table 2.1: Analysis of variance for differences in performance measures among habitats, among locations and among populations (habitat*location).

*** Highly significant ($p < 0.001$); ** Significant ($0.01 < p < 0.001$); * Slightly significant ($0.05 < p < 0.01$); ns, not significant.

Multiple pairwise comparisons of the population means using Tukey's HSD test were performed to determine which specific population means differ from which others. This method creates a set of confidence intervals, based on the null hypothesis that the difference in the means is zero, with the specified (95 %) family-wise probability of coverage. The Tukey's HSD tests performed on each of the performance measures found that there were no significant differences between any of the population mean comparisons. The Tukey's HSD plots show these results clearly; if the confidence interval line intersects zero, the population means are not significantly different (Figure 2.3–2.9). The performance trait, number of

flowering stems, showed a slightly significant ANOVA result ($p = 0.0422$) for the habitat*location interaction, suggesting that at least one of the population means is significantly different from the others. The Tukey's HSD test for the number of flowering stems showed that there were in fact no significant differences among the population means; the confidence intervals for every population mean comparison intersect zero and have non-significant p-values (Figure 2.4). This indicates that there is no significant interpopulation variation in the number of flowering stems produced.

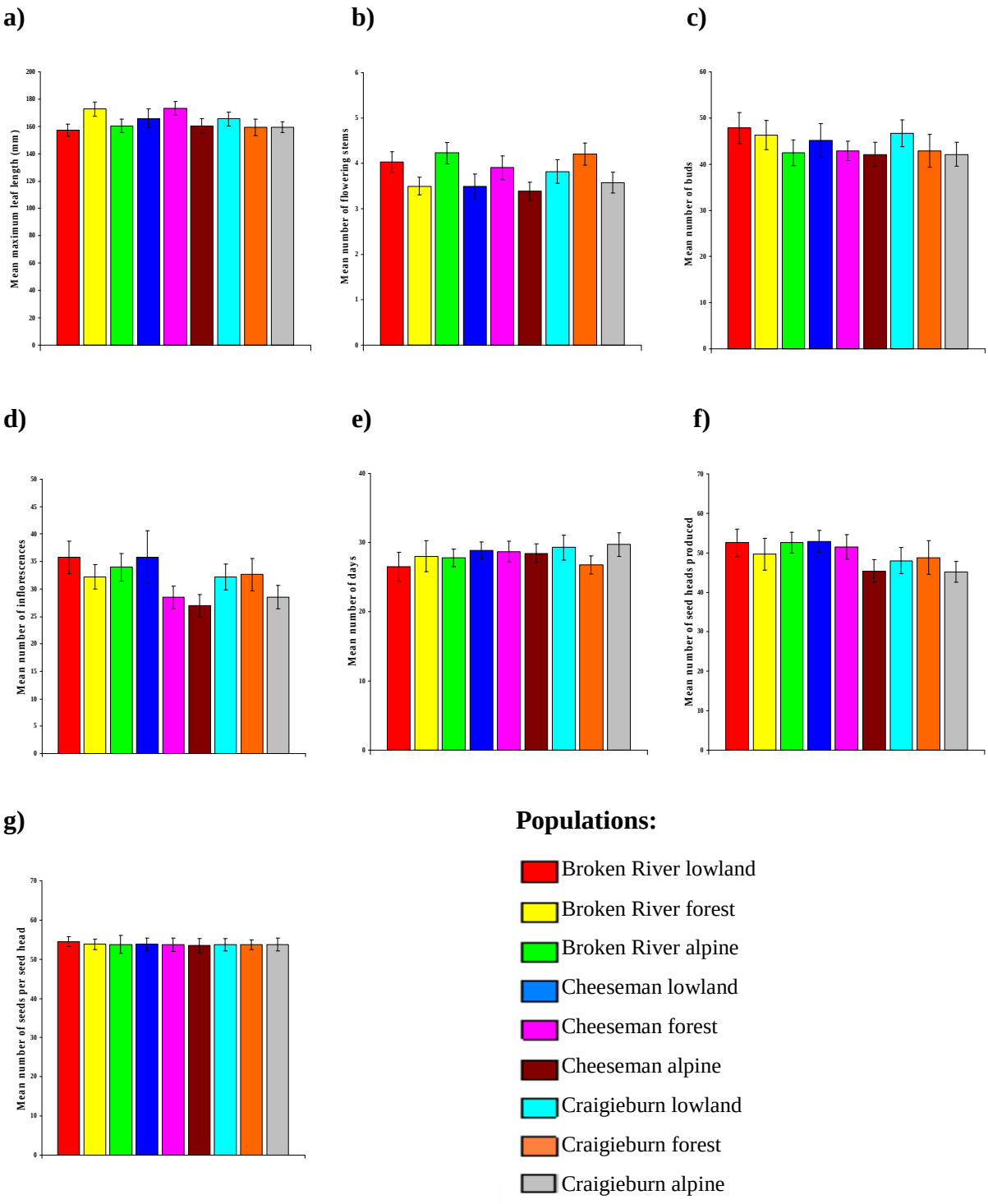
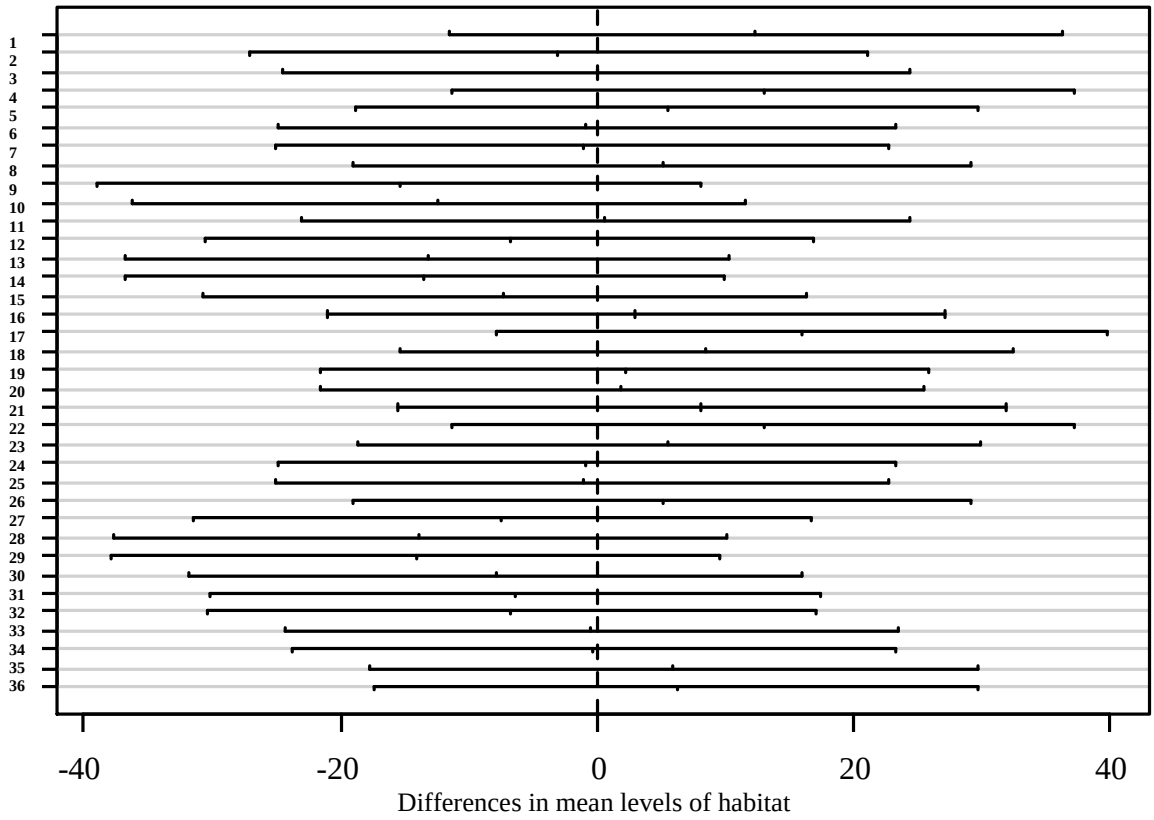


Figure 2.2: Means for each population for the performance measures; (a) maximum leaf length (mm), (b) number of flowering stems, (c) number of buds, (d) number of inflorescences, (e) days until first flower, (f) total number of seed heads produced, and (g) seeds per seed head for the *Hieracium lepidulum* populations grown in the common garden. Vertical bars denote the standard error of the mean.

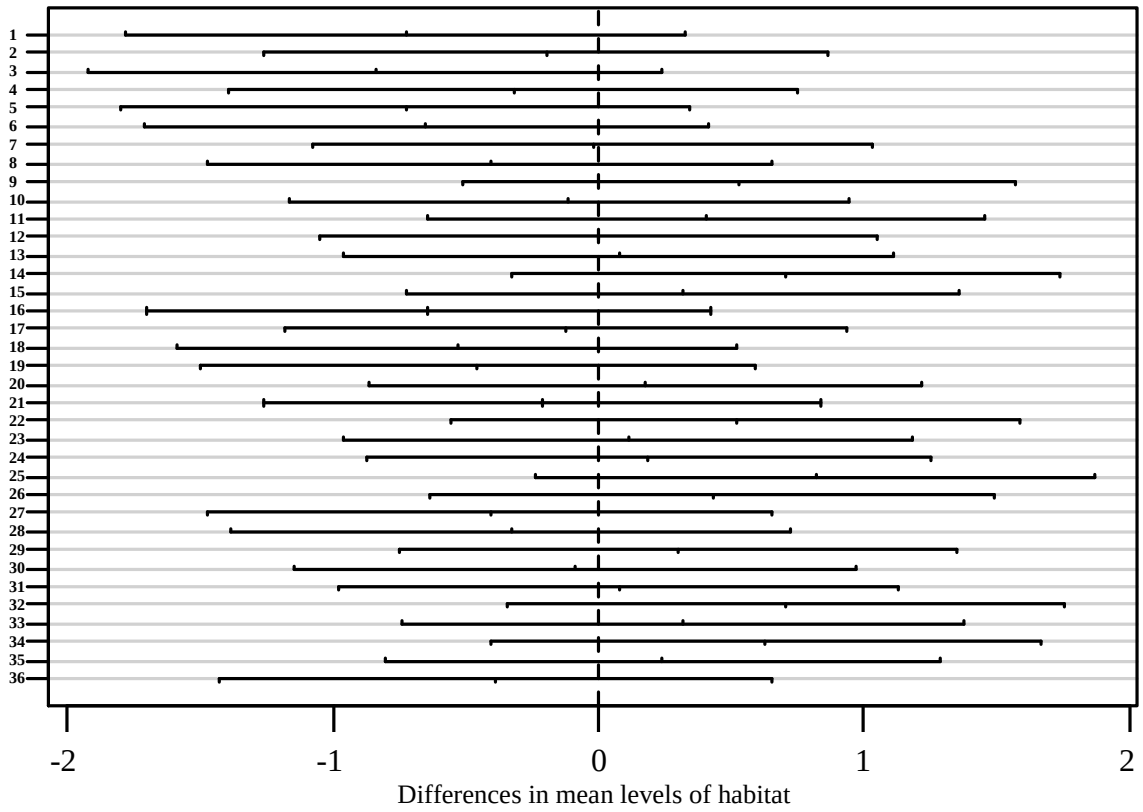


Key:

	Comparison	p value		Comparison	p value
1 -	Broken River forest - Broken River alpine	(0.798)	19 -	Craigieburn alpine - Broken River lowland	(1.000)
2 -	Broken River lowland - Broken River alpine	(1.000)	20 -	Craigieburn forest - Broken River lowland	(1.000)
3 -	Cheeseman alpine - Broken River alpine	(1.000)	21 -	Craigieburn lowland - Broken River lowland	(0.978)
4 -	Cheeseman forest - Broken River alpine	(0.764)	22 -	Cheeseman forest - Cheeseman alpine	(0.762)
5 -	Cheeseman lowland - Broken River alpine	(0.999)	23 -	Cheeseman lowland - Cheeseman alpine	(0.999)
6 -	Craigieburn alpine - Broken River alpine	(1.000)	24 -	Craigieburn alpine - Cheeseman alpine	(1.000)
7 -	Craigieburn forest - Broken River alpine	(1.000)	25 -	Craigieburn forest - Cheeseman alpine	(1.000)
8 -	Craigieburn lowland - Broken River alpine	(0.999)	26 -	Craigieburn lowland - Cheeseman alpine	(0.999)
9 -	Broken River lowland - Broken River forest	(0.517)	27 -	Cheeseman lowland - Cheeseman forest	(0.989)
10 -	Cheeseman alpine - Broken River forest	(0.796)	28 -	Craigieburn alpine - Cheeseman forest	(0.678)
11 -	Cheeseman forest - Broken River forest	(1.000)	29 -	Craigieburn forest - Cheeseman forest	(0.644)
12 -	Cheeseman lowland - Broken River forest	(0.993)	30 -	Craigieburn lowland - Cheeseman forest	(0.983)
13 -	Craigieburn alpine - Broken River forest	(0.715)	31 -	Craigieburn alpine - Cheeseman lowland	(0.996)
14 -	Craigieburn forest - Broken River forest	(0.682)	32 -	Craigieburn forest - Cheeseman lowland	(0.994)
15 -	Craigieburn lowland - Broken River forest	(0.989)	33 -	Craigieburn lowland - Cheeseman lowland	(1.000)
16 -	Cheeseman alpine - Broken River lowland	(1.000)	34 -	Craigieburn forest - Craigieburn alpine	(1.000)
17 -	Cheeseman forest - Broken River lowland	(0.481)	35 -	Craigieburn lowland - Craigieburn alpine	(0.997)
18 -	Cheeseman lowland - Broken River lowland	(0.971)	36 -	Craigieburn lowland - Craigieburn forest	(0.996)

Figure 2.3: 95 % family-wise confidence level plot for maximum leaf length.

Each horizontal plot-line represents the comparison of two population means (refer to key; the p value, after adjustment from multiple comparisons, is given for each comparison. Significant p values are indicated by a *). The central tick-mark on the line denotes the difference between the two means, with the upper and lower bounds of the confidence intervals indicated by the end of the plot-line. Population means are significantly different if the plot-line does not intersect zero.

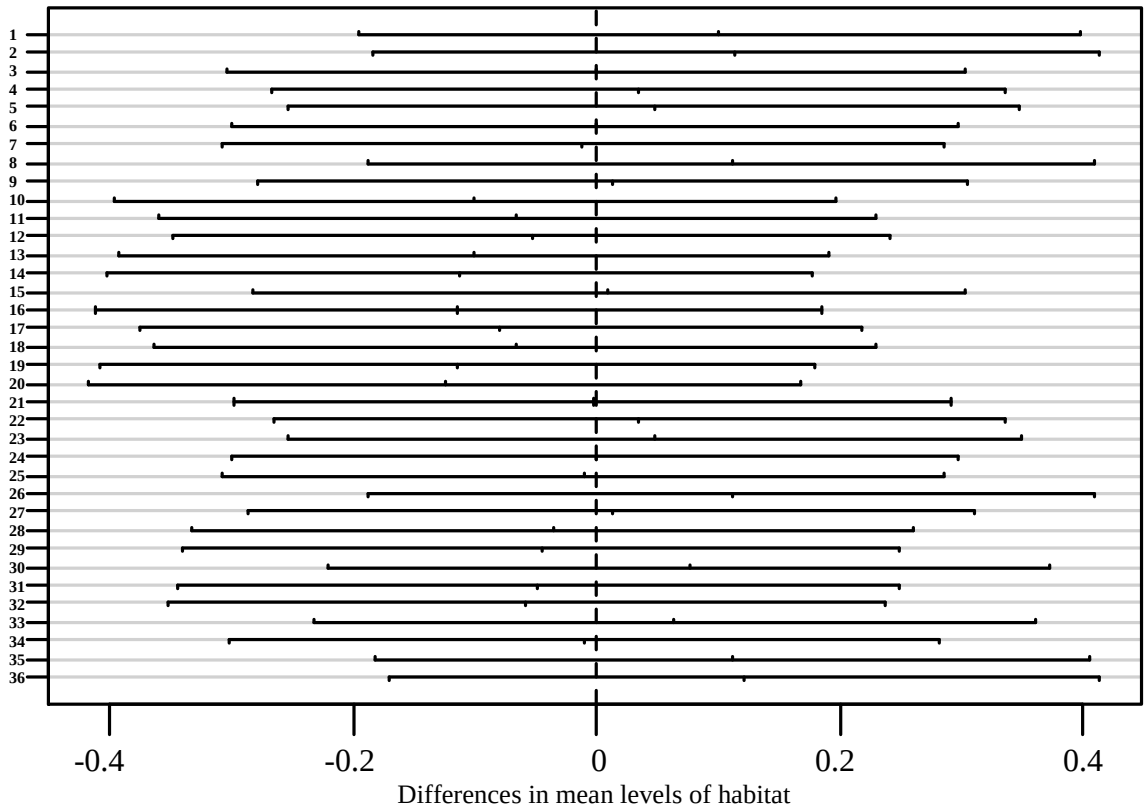


Key:

	Comparison	p value		Comparison	p value
1 -	Broken River forest - Broken River alpine	(0.442)	19 -	Craigieburn alpine - Broken River lowland	(0.913)
2 -	Broken River lowland - Broken River alpine	(0.997)	20 -	Craigieburn forest - Broken River lowland	(1.000)
3 -	Cheeseman alpine - Broken River alpine	(0.273)	21 -	Craigieburn lowland - Broken River lowland	(0.999)
4 -	Cheeseman forest - Broken River alpine	(0.273)	22 -	Cheeseman forest - Cheeseman alpine	(0.848)
5 -	Cheeseman lowland - Broken River alpine	(0.464)	23 -	Cheeseman lowland - Cheeseman alpine	(1.000)
6 -	Craigieburn alpine - Broken River alpine	(0.607)	24 -	Craigieburn alpine - Cheeseman alpine	(0.998)
7 -	Craigieburn forest - Broken River alpine	(1.000)	25 -	Craigieburn forest - Cheeseman alpine	(0.275)
8 -	Craigieburn lowland - Broken River alpine	(0.956)	26 -	Craigieburn lowland - Cheeseman alpine	(0.940)
9 -	Broken River lowland - Broken River forest	(0.807)	27 -	Cheeseman lowland - Cheeseman forest	(0.957)
10 -	Cheeseman alpine - Broken River forest	(1.000)	28 -	Craigieburn alpine - Cheeseman forest	(0.987)
11 -	Cheeseman forest - Broken River forest	(0.953)	29 -	Craigieburn forest - Cheeseman forest	(0.993)
12 -	Cheeseman lowland - Broken River forest	(1.000)	30 -	Craigieburn lowland - Cheeseman forest	(1.000)
13 -	Craigieburn alpine - Broken River forest	(1.000)	31 -	Craigieburn alpine - Cheeseman lowland	(1.000)
14 -	Craigieburn forest - Broken River forest	(0.449)	32 -	Craigieburn forest - Cheeseman lowland	(0.471)
15 -	Craigieburn lowland - Broken River forest	(0.989)	33 -	Craigieburn lowland - Cheeseman lowland	(0.990)
16 -	Cheeseman alpine - Broken River lowland	(0.621)	34 -	Craigieburn forest - Craigieburn alpine	(0.617)
17 -	Cheeseman forest - Broken River lowland	(1.000)	35 -	Craigieburn lowland - Craigieburn alpine	(0.998)
18 -	Cheeseman lowland - Broken River lowland	(0.820)	36 -	Craigieburn lowland - Craigieburn forest	(0.963)

Figure 2.4: 95 % family-wise confidence level plot for number of flowering stems.

Each horizontal plot-line represents the comparison of two population means (refer to key; the p value, after adjustment from multiple comparisons, is given for each comparison. Significant p values are indicated by a *). The central tick-mark on the line denotes the difference between the two means, with the upper and lower bounds of the confidence intervals indicated by the end of the plot-line. Population means are significantly different if the plot-line does not intersect zero.

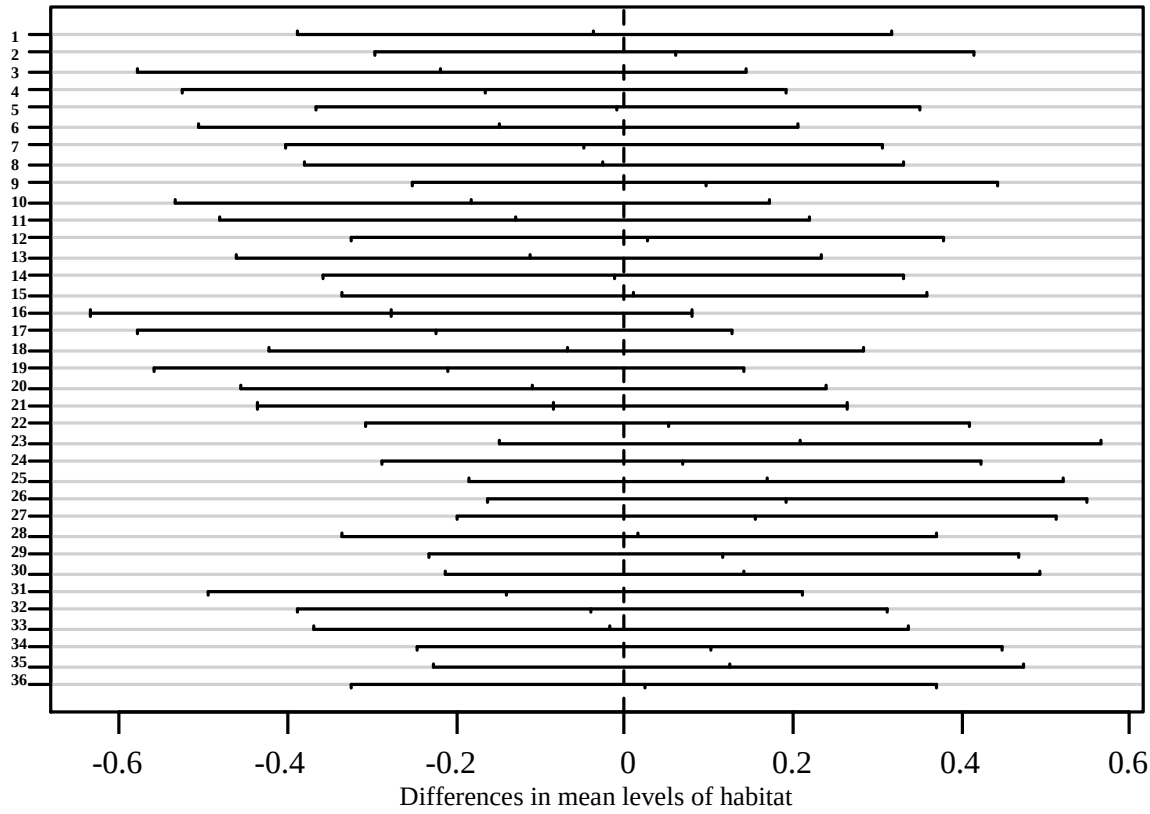


Key:

	Comparison	p value		Comparison	p value
1 -	Broken River forest - Broken River alpine	(0.979)	19 -	Craigieburn alpine - Broken River lowland	(0.918)
2 -	Broken River lowland - Broken River alpine	(0.958)	20 -	Craigieburn forest - Broken River lowland	(1.000)
3 -	Cheeseman alpine - Broken River alpine	(1.000)	21 -	Craigieburn lowland - Broken River lowland	(1.000)
4 -	Cheeseman forest - Broken River alpine	(1.000)	22 -	Cheeseman forest - Cheeseman alpine	(1.000)
5 -	Cheeseman lowland - Broken River alpine	(1.000)	23 -	Cheeseman lowland - Cheeseman alpine	(1.000)
6 -	Craigieburn alpine - Broken River alpine	(1.000)	24 -	Craigieburn alpine - Cheeseman alpine	(1.000)
7 -	Craigieburn forest - Broken River alpine	(1.000)	25 -	Craigieburn forest - Cheeseman alpine	(1.000)
8 -	Craigieburn lowland - Broken River alpine	(0.965)	26 -	Craigieburn lowland - Cheeseman alpine	(0.964)
9 -	Broken River lowland - Broken River forest	(1.000)	27 -	Cheeseman lowland - Cheeseman forest	(1.000)
10 -	Cheeseman alpine - Broken River forest	(0.979)	28 -	Craigieburn alpine - Cheeseman forest	(1.000)
11 -	Cheeseman forest - Broken River forest	(0.999)	29 -	Craigieburn forest - Cheeseman forest	(1.000)
12 -	Cheeseman lowland - Broken River forest	(1.000)	30 -	Craigieburn lowland - Cheeseman forest	(1.000)
13 -	Craigieburn alpine - Broken River forest	(0.975)	31 -	Craigieburn alpine - Cheeseman lowland	(1.000)
14 -	Craigieburn forest - Broken River forest	(0.954)	32 -	Craigieburn forest - Cheeseman lowland	(1.000)
15 -	Craigieburn lowland - Broken River forest	(1.000)	33 -	Craigieburn lowland - Cheeseman lowland	(0.990)
16 -	Cheeseman alpine - Broken River lowland	(0.958)	34 -	Craigieburn forest - Craigieburn alpine	(1.000)
17 -	Cheeseman forest - Broken River lowland	(0.996)	35 -	Craigieburn lowland - Craigieburn alpine	(0.959)
18 -	Cheeseman lowland - Broken River lowland	(0.999)	36 -	Craigieburn lowland - Craigieburn forest	(0.930)

Figure 2.5: 95 % family-wise confidence level plot for number of buds.

Each horizontal plot-line represents the comparison of two population means (refer to key; the p value, after adjustment from multiple comparisons, is given for each comparison. Significant p values are indicated by a *). The central tick-mark on the line denotes the difference between the two means, with the upper and lower bounds of the confidence intervals indicated by the end of the plot-line. Population means are significantly different if the plot-line does not intersect zero.

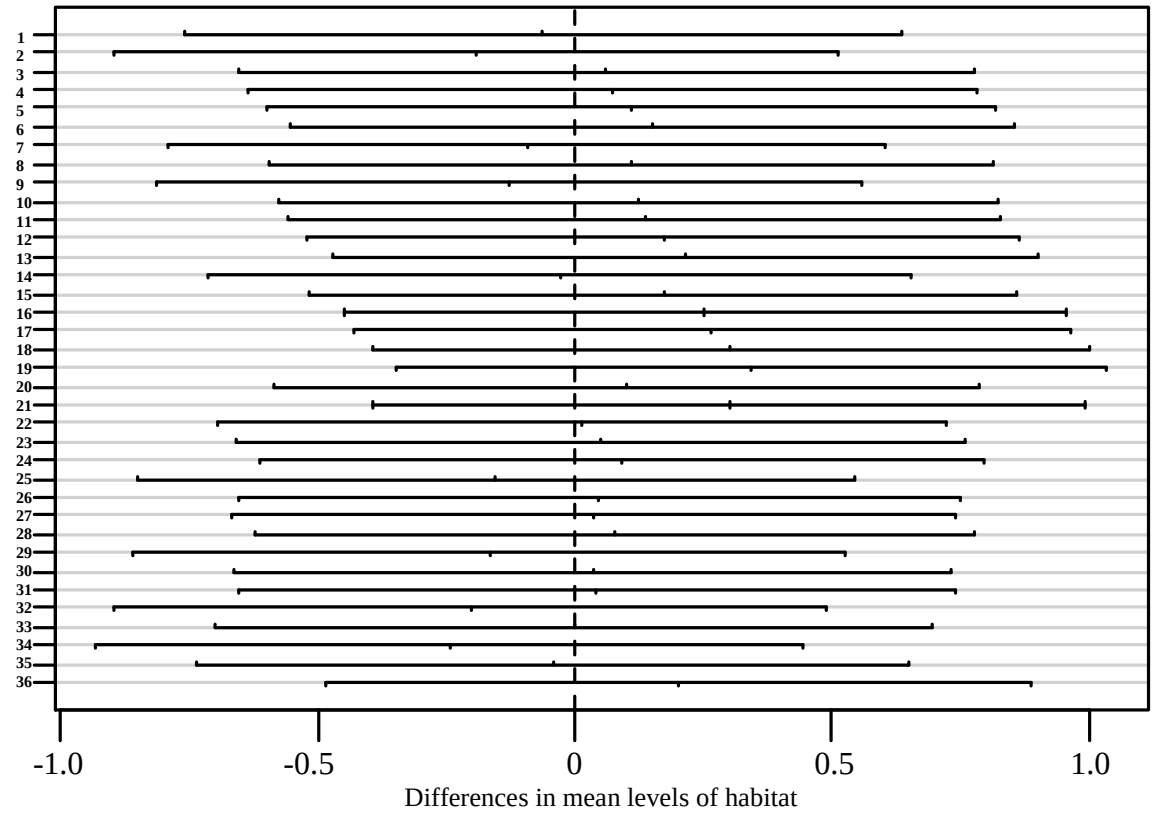


Key:

	Comparison	p value		Comparison	p value
1 -	Broken River forest - Broken River alpine	(1.000)	19 -	Craigieburn alpine - Broken River lowland	(0.635)
2 -	Broken River lowland - Broken River alpine	(1.000)	20 -	Craigieburn forest - Broken River lowland	(0.988)
3 -	Cheeseman alpine - Broken River alpine	(0.628)	21 -	Craigieburn lowland - Broken River lowland	(0.998)
4 -	Cheeseman forest - Broken River alpine	(0.880)	22 -	Cheeseman forest - Cheeseman alpine	(1.000)
5 -	Cheeseman lowland - Broken River alpine	(1.000)	23 -	Cheeseman lowland - Cheeseman alpine	(0.671)
6 -	Craigieburn alpine - Broken River alpine	(0.926)	24 -	Craigieburn alpine - Cheeseman alpine	(1.000)
7 -	Craigieburn forest - Broken River alpine	(1.000)	25 -	Craigieburn forest - Cheeseman alpine	(0.859)
8 -	Craigieburn lowland - Broken River alpine	(1.000)	26 -	Craigieburn lowland - Cheeseman alpine	(0.755)
9 -	Broken River lowland - Broken River forest	(0.995)	27 -	Cheeseman lowland - Cheeseman forest	(0.906)
10 -	Cheeseman alpine - Broken River forest	(0.801)	28 -	Craigieburn alpine - Cheeseman forest	(1.000)
11 -	Cheeseman forest - Broken River forest	(0.965)	29 -	Craigieburn forest - Cheeseman forest	(0.981)
12 -	Cheeseman lowland - Broken River forest	(1.000)	30 -	Craigieburn lowland - Cheeseman forest	(0.946)
13 -	Craigieburn alpine - Broken River forest	(0.983)	31 -	Craigieburn alpine - Cheeseman lowland	(0.945)
14 -	Craigieburn forest - Broken River forest	(1.000)	32 -	Craigieburn forest - Cheeseman lowland	(1.000)
15 -	Craigieburn lowland - Broken River forest	(1.000)	33 -	Craigieburn lowland - Cheeseman lowland	(1.000)
16 -	Cheeseman alpine - Broken River lowland	(0.270)	34 -	Craigieburn forest - Craigieburn alpine	(0.992)
17 -	Cheeseman forest - Broken River lowland	(0.547)	35 -	Craigieburn lowland - Craigieburn alpine	(0.972)
18 -	Cheeseman lowland - Broken River lowland	(1.000)	36 -	Craigieburn lowland - Craigieburn forest	(1.000)

Figure 2.6: 95 % family-wise confidence level plot for number of inflorescences.

Each horizontal plot-line represents the comparison of two population means (refer to key; the p value, after adjustment from multiple comparisons, is given for each comparison. Significant p values are indicated by a *). The central tick-mark on the line denotes the difference between the two means, with the upper and lower bounds of the confidence intervals indicated by the end of the plot-line. Population means are significantly different if the plot-line does not intersect zero.

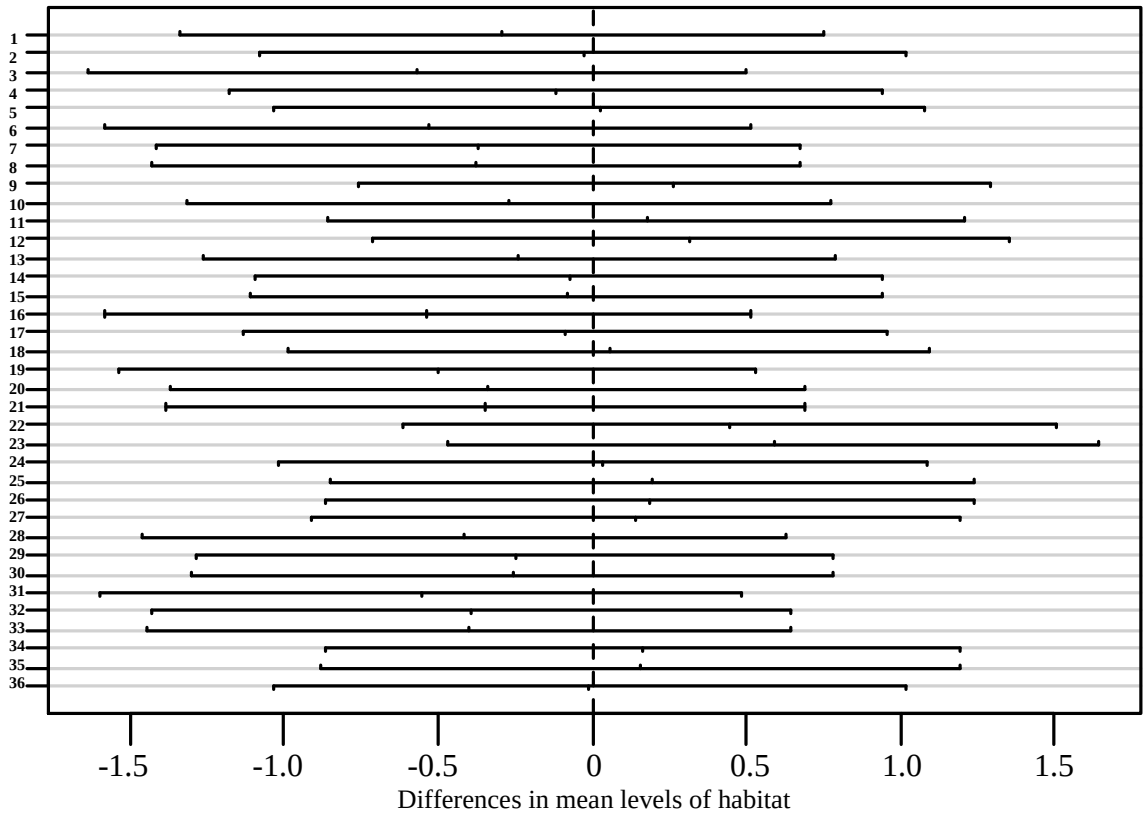


Key:

Comparison	p value	Comparison	p value
1 - Broken River forest - Broken River alpine	(1.000)	19 - Craigieburn alpine - Broken River lowland	(0.831)
2 - Broken River lowland - Broken River alpine	(0.995)	20 - Craigieburn forest - Broken River lowland	(0.913)
3 - Cheeseman alpine - Broken River alpine	(1.000)	21 - Craigieburn lowland - Broken River lowland	(0.913)
4 - Cheeseman forest - Broken River alpine	(1.000)	22 - Cheeseman forest - Cheeseman alpine	(1.000)
5 - Cheeseman lowland - Broken River alpine	(1.000)	23 - Cheeseman lowland - Cheeseman alpine	(1.000)
6 - Craigieburn alpine - Broken River alpine	(0.999)	24 - Craigieburn alpine - Cheeseman alpine	(1.000)
7 - Craigieburn forest - Broken River alpine	(1.000)	25 - Craigieburn forest - Cheeseman alpine	(0.999)
8 - Craigieburn lowland - Broken River alpine	(1.000)	26 - Craigieburn lowland - Cheeseman alpine	(1.000)
9 - Broken River lowland - Broken River forest	(1.000)	27 - Cheeseman lowland - Cheeseman forest	(1.000)
10 - Cheeseman alpine - Broken River forest	(0.988)	28 - Craigieburn alpine - Cheeseman forest	(1.000)
11 - Cheeseman forest - Broken River forest	(1.000)	29 - Craigieburn forest - Cheeseman forest	(0.998)
12 - Cheeseman lowland - Broken River forest	(0.997)	30 - Craigieburn lowland - Cheeseman forest	(1.000)
13 - Craigieburn alpine - Broken River forest	(0.988)	31 - Craigieburn alpine - Cheeseman lowland	(1.000)
14 - Craigieburn forest - Broken River forest	(1.000)	32 - Craigieburn forest - Cheeseman lowland	(0.992)
15 - Craigieburn lowland - Broken River forest	(0.997)	33 - Craigieburn lowland - Cheeseman lowland	(1.000)
16 - Cheeseman alpine - Broken River lowland	(0.970)	34 - Craigieburn forest - Craigieburn alpine	(0.973)
17 - Cheeseman forest - Broken River lowland	(0.958)	35 - Craigieburn lowland - Craigieburn alpine	(1.000)
18 - Cheeseman lowland - Broken River lowland	(0.914)	36 - Craigieburn lowland - Craigieburn forest	(0.992)

Figure 2.7: 95 % family-wise confidence level plot for days until first flower.

Each horizontal plot-line represents the comparison of two population means (refer to key; the p value, after adjustment from multiple comparisons, is given for each comparison. Significant p values are indicated by a *). The central tick-mark on the line denotes the difference between the two means, with the upper and lower bounds of the confidence intervals indicated by the end of the plot-line. Population means are significantly different if the plot-line does not intersect zero.

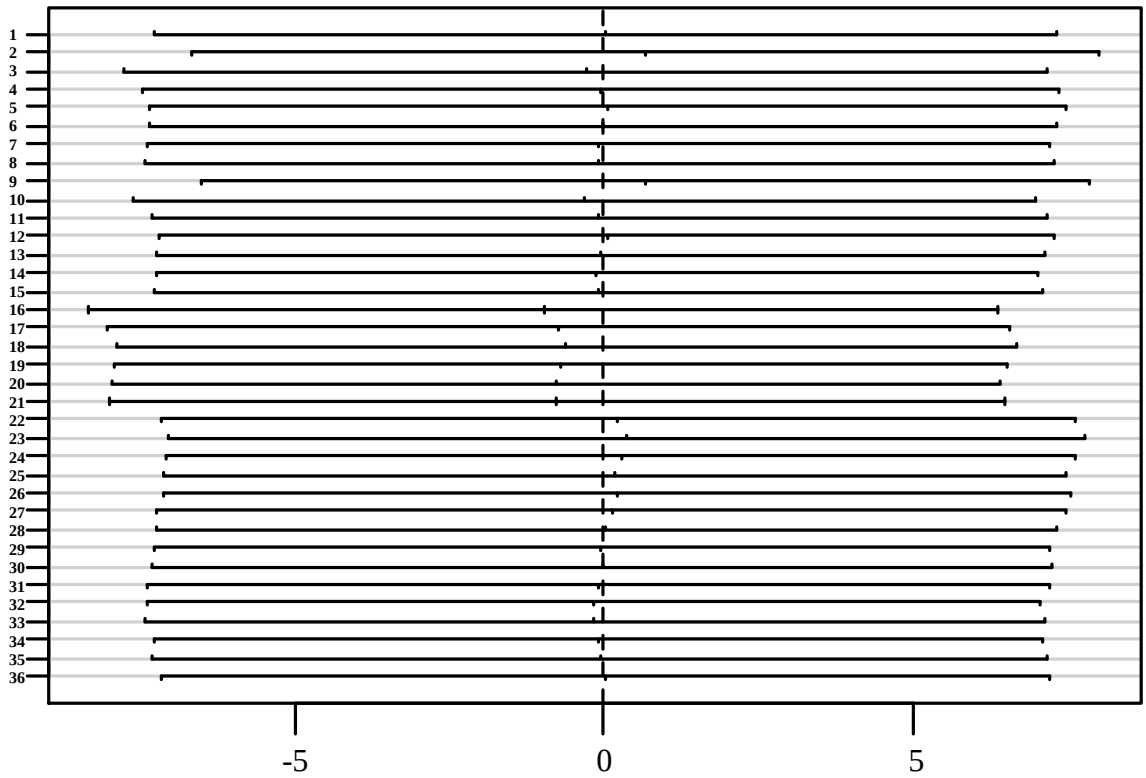


Key:

	Comparison	p value		Comparison	p value
1 -	Broken River forest - Broken River alpine	(0.994)	19 -	Craigieburn alpine - Broken River lowland	(0.845)
2 -	Broken River lowland - Broken River alpine	(1.000)	20 -	Craigieburn forest - Broken River lowland	(0.982)
3 -	Cheeseman alpine - Broken River alpine	(0.773)	21 -	Craigieburn lowland - Broken River lowland	(0.980)
4 -	Cheeseman forest - Broken River alpine	(1.000)	22 -	Cheeseman forest - Cheeseman alpine	(0.925)
5 -	Cheeseman lowland - Broken River alpine	(1.000)	23 -	Cheeseman lowland - Cheeseman alpine	(0.723)
6 -	Craigieburn alpine - Broken River alpine	(0.812)	24 -	Craigieburn alpine - Cheeseman alpine	(1.000)
7 -	Craigieburn forest - Broken River alpine	(0.970)	25 -	Craigieburn forest - Cheeseman alpine	(1.000)
8 -	Craigieburn lowland - Broken River alpine	(0.970)	26 -	Craigieburn lowland - Cheeseman alpine	(1.000)
9 -	Broken River lowland - Broken River forest	(0.997)	27 -	Cheeseman lowland - Cheeseman forest	(1.000)
10 -	Cheeseman alpine - Broken River forest	(0.997)	28 -	Craigieburn alpine - Cheeseman forest	(0.946)
11 -	Cheeseman forest - Broken River forest	(1.000)	29 -	Craigieburn forest - Cheeseman forest	(0.998)
12 -	Cheeseman lowland - Broken River forest	(0.989)	30 -	Craigieburn lowland - Cheeseman forest	(0.997)
13 -	Craigieburn alpine - Broken River forest	(0.998)	31 -	Craigieburn alpine - Cheeseman lowland	(0.765)
14 -	Craigieburn forest - Broken River forest	(1.000)	32 -	Craigieburn forest - Cheeseman lowland	(0.959)
15 -	Craigieburn lowland - Broken River forest	(1.000)	33 -	Craigieburn lowland - Cheeseman lowland	(0.956)
16 -	Cheeseman alpine - Broken River lowland	(0.809)	34 -	Craigieburn forest - Craigieburn alpine	(1.000)
17 -	Cheeseman forest - Broken River lowland	(1.000)	35 -	Craigieburn lowland - Craigieburn alpine	(1.000)
18 -	Cheeseman lowland - Broken River lowland	(1.000)	36 -	Craigieburn lowland - Craigieburn forest	(1.000)

Figure 2.8: 95 % family-wise confidence level plot for total number of seed heads.

Each horizontal plot-line represents the comparison of two population means (refer to key; the p value, after adjustment from multiple comparisons, is given for each comparison. Significant p values are indicated by a *). The central tick-mark on the line denotes the difference between the two means, with the upper and lower bounds of the confidence intervals indicated by the end of the plot-line. Population means are significantly different if the plot-line does not intersect zero.



Key:

	Comparison	p value		Comparison	p value
1 -	Broken River forest - Broken River alpine	(1.000)	19 -	Craigieburn alpine - Broken River lowland	(1.000)
2 -	Broken River lowland - Broken River alpine	(1.000)	20 -	Craigieburn forest - Broken River lowland	(1.000)
3 -	Cheeseman alpine - Broken River alpine	(1.000)	21 -	Craigieburn lowland - Broken River lowland	(1.000)
4 -	Cheeseman forest - Broken River alpine	(1.000)	22 -	Cheeseman forest - Cheeseman alpine	(1.000)
5 -	Cheeseman lowland - Broken River alpine	(1.000)	23 -	Cheeseman lowland - Cheeseman alpine	(1.000)
6 -	Craigieburn alpine - Broken River alpine	(1.000)	24 -	Craigieburn alpine - Cheeseman alpine	(1.000)
7 -	Craigieburn forest - Broken River alpine	(1.000)	25 -	Craigieburn forest - Cheeseman alpine	(1.000)
8 -	Craigieburn lowland - Broken River alpine	(1.000)	26 -	Craigieburn lowland - Cheeseman alpine	(1.000)
9 -	Broken River lowland - Broken River forest	(1.000)	27 -	Cheeseman lowland - Cheeseman forest	(1.000)
10 -	Cheeseman alpine - Broken River forest	(1.000)	28 -	Craigieburn alpine - Cheeseman forest	(1.000)
11 -	Cheeseman forest - Broken River forest	(1.000)	29 -	Craigieburn forest - Cheeseman forest	(1.000)
12 -	Cheeseman lowland - Broken River forest	(1.000)	30 -	Craigieburn lowland - Cheeseman forest	(1.000)
13 -	Craigieburn alpine - Broken River forest	(1.000)	31 -	Craigieburn alpine - Cheeseman lowland	(1.000)
14 -	Craigieburn forest - Broken River forest	(1.000)	32 -	Craigieburn forest - Cheeseman lowland	(1.000)
15 -	Craigieburn lowland - Broken River forest	(1.000)	33 -	Craigieburn lowland - Cheeseman lowland	(1.000)
16 -	Cheeseman alpine - Broken River lowland	(1.000)	34 -	Craigieburn forest - Craigieburn alpine	(1.000)
17 -	Cheeseman forest - Broken River lowland	(1.000)	35 -	Craigieburn lowland - Craigieburn alpine	(1.000)
18 -	Cheeseman lowland - Broken River lowland	(1.000)	36 -	Craigieburn lowland - Craigieburn forest	(1.000)

Figure 2.9: 95 % family-wise confidence level plot for seeds per seed head.

Each horizontal plot-line represents the comparison of two population means (refer to key; the p value, after adjustment from multiple comparisons, is given for each comparison. Significant p values are indicated by a *). The central tick-mark on the line denotes the difference between the two means, with the upper and lower bounds of the confidence intervals indicated by the end of the plot-line. Population means are significantly different if the plot-line does not intersect zero.

2.4 Discussion

From a previous study (Miller 2005) on *Hieracium lepidulum* in the Craigieburn Range, we know that populations occupying different habitats display marked phenotypic differences. Overall Miller (2005) demonstrated that the alpine habitats had a higher level of plant performance than the forest habitats. Leaf lengths were longer in the alpine habitat, therefore plants were larger in size, and plants in the alpine habitats were also more fecund. Plants growing in the low-lying grassland, ~750 m altitude, tend to form dense meadows (Chapman *et al.* 2004), with overall plant size reduced (pers. obs.). The purpose of the common garden experiment was to determine if these phenotypic differences observed among habitats in the field were a result of phenotypic plasticity or selection and adaptation. If this phenotypic variation is a result of selection and adaptation, the populations in the common garden should also exhibit these phenotypic differences as they reflect the genotype. Selection will favour individuals with traits most suited to the habitat and those genotypes will prosper, leading to genetically-distinct populations among habitats. If the performance measures show no significant variation among the populations in the common garden it suggests the phenotypic variation found in the field is due to plasticity. The common garden experiment determined that populations from different habitats in the Craigieburn Range are not genetically distinct; they possess a 'general-purpose genotype' which provides the ability to prosper across a variable environmental gradient.

The common garden experiment confirmed that populations of *Hieracium lepidulum*, sampled from different habitats and locations in the Craigieburn Range, are in fact plastic. None of the performance measures showed significant statistical differences among the three

habitats (‘lowland’, ‘forest’ and ‘alpine’). This implies that plasticity, rather than selection and adaptation, is the source of the phenotypic differences observed among habitats in the field; variable phenotypes are produced in response to the different environmental pressures in each habitat rather than due to genetic variation.

The mode of reproduction in this species can explain the common garden findings of no genetic variation among habitats. *H. lepidulum* is an obligate apomict. It reproduces through the production of clonal seed which does not involve meiosis or fertilisation, and is essentially the complete transmission of the entire maternal genotype to the offspring (Kultunow *et al.* 1995, Chapman *et al*. 2004). Apomixis fixes a particular genotype, and apomicts are therefore described as clonal lineages (Kultunow *et al.* 1995, Storchova *et al*. 2002, Chapman *et al*. 2004). This mechanism of reproduction results in a colossal number of asexual clones, and populations should harbour very low levels of genetic variation (Bayer *et al.* 1990, Storchova *et al*. 2002, Chapman *et al*. 2004). There have been no recordings of sexual diploids in *H. lepidulum*, so intraspecific hybridisation as a means of providing genetic variation is very unlikely (Chapman *et al*. 2004). Without genetic variation, natural selection has nothing on which to act. By considering the breeding system and these common garden results, there is no evidence to support the mechanism of selection and adaptation as a means of producing phenotypic variation. Phenotypic plasticity must be the process responsible for the phenotypic variation observed among habitats in the Craigieburn Range.

There were also no significant statistical differences in the performance traits among the three locations that *H. lepidulum* was sampled from or among any of the nine populations. From these results it can be deduced that the populations in this study are genetically similar. This indicates that the populations originate from the same clonal lineage and there are no barriers for dispersal throughout the three locations, Broken River, Cheeseman and Craigieburn. This is supported by herbarium data which shows the first recorded occurrence of *Hieracium lepidulum* in New Zealand was from the Craigieburn Range where it is thought to have acted as a centre of spread for the species (Miller 2005).

2.5 Summary

Populations of *Hieracium lepidulum* occupying different habitats in the Craigieburn Range show distinct phenotypic variation (Miller 2005). For the common garden experiment populations were sampled from three replicate altitudes within three geographically-separate locations. The objective of this experiment was to determine whether the phenotypic variation seen in the field is a result of phenotypic plasticity or genetic variation via selection and adaptation. Plant performance traits showed no significant statistical differences:

1) Among habitats. This suggests there is no genetic variation for these performance traits among habitats, consequently excluding the mechanism of selection and adaptation, as selection requires genetic variation on which to act. This confirms the mechanism of phenotypic plasticity, the production of multiple phenotypes from a single genotype in response to environmental conditions.

2) Among locations. This implies there is no genetic variability for these performance measures among the three locations, suggesting that the populations all originated from the same source population, with no dispersal barriers.

3) Among populations. This indicates there is no genetic variation among all nine populations for the performance traits measured here; it is likely populations belong to the same clonal lineage.

Chapter 3

3. Phenotypic plasticity in response to environmental stress

3.1 Introduction

Hieracium lepidulum is a major threat to the indigenous flora of New Zealand. The invasion success of *H. lepidulum* is due in part to its great ability in colonising a wide range of habitats, including; pine plantations, scrubland, native *Nothofagus* forest, and mid-altitude to alpine tussock grassland (from 750 m to 1700 m in altitude) (Treskonova 1991, Rose *et al*. 1995, Duncan *et al*. 1997, Chapman *et al*. 2004). Common garden experiments (Chapter 2) have found that phenotypic plasticity is the mechanism responsible for this species' ability to produce variable phenotypes in heterogeneous environments.

Phenotypic plasticity is the ability of a single genotype to develop multiple phenotypic states depending on the environmental conditions (Barrett 1982, Miner *et al*. 2005). Plastic responses to the environment can include; changes in morphology, physiology, behaviour, life history, growth, and demography (Miner *et al*. 2005). Plastic responses can occur within the lifespan of an individual or across generations (Miner *et al*. 2005). Plasticity is thought to be very important in the process of invasion as it allows the exotic to respond within a single generation to their new environment to escape a decrease in fitness (Sakai *et al*. 2001, Bossdorf *et al*. 2005). Phenotypic plasticity would bestow a fitness advantage for an invading species which suffered from a lack of genetic variation preventing adaptation via natural selection (Bossdorf *et al*. 2005).

Phenotypic plasticity is essentially controlled at the genetic level; a single genotype can express multiple phenotypes in variable environments. Studies have found the amount and pattern of genetic variation that is expressed can be environment dependent (i.e. genetic variation for plasticity among habitats) and that there is also genetic variation for plasticity within populations (Pigliucci *et al*. 1995, Volis *et al*. 2002, Pigliucci 2005 and refs. within, Kovnat 2007, Bell and Galloway 2008). As variation is a necessity for natural selection, the plastic expression of a genotype could therefore evolve due to variation in the loci expressing the plastic phenotypes, resulting in adaptive phenotypic plasticity occurring in natural populations (Pigliucci *et al*. 1995, Pigliucci 2005, Bell and Galloway 2008). A way to determine if plasticity is adaptive is by measuring selection on phenotypic traits within certain environments; if selection differs between environments and the plastic response within environments is in the same direction as selection, the plasticity is adaptive (Bell and Galloway 2008).

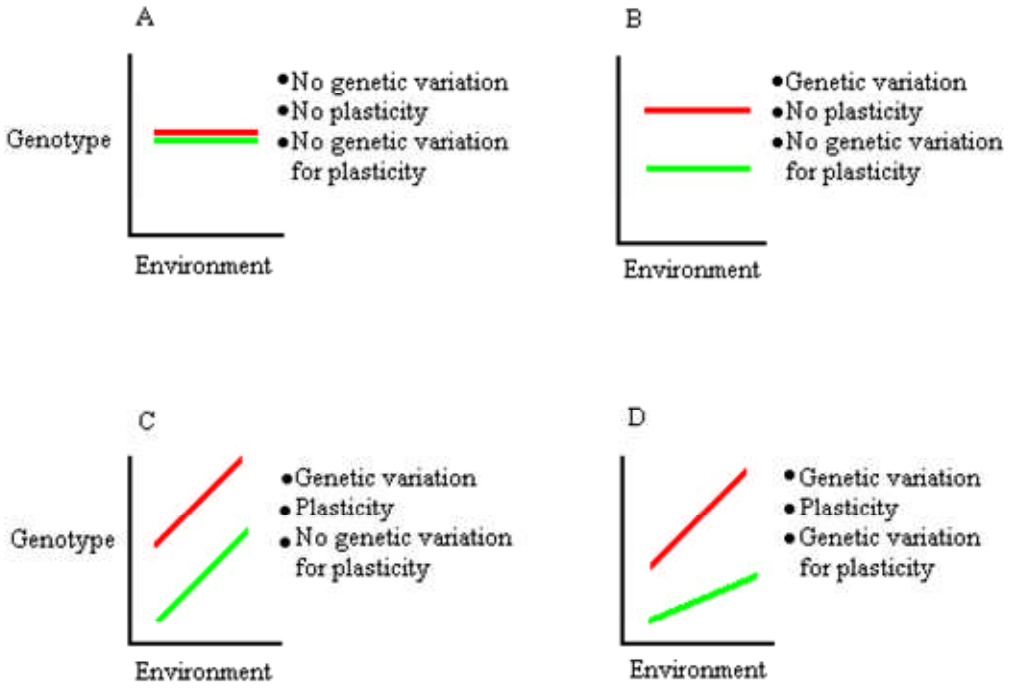
3.1.1 Objective two

The aim of this section is to investigate the genetic variation, phenotypic plasticity and genetic variability for plasticity in populations of *Hieracium lepidulum* subjected to two types of abiotic 'stress', shade and drought (compared to an 'optimal' control).

3.2 Methods

3.2.1 Experimental background

The range of plasticity within and among populations under different environments can be expressed using reaction norms. A reaction norm is a set of phenotypes produced by a genotype in response to different environments (Pigliucci *et al*. 1995, Volis *et al*. 2002, Miner *et al*. 2005). Phenotypic plasticity is the degree and direction of departure of the reaction norm from a parallel to the environmental axis flat line (Pigliucci *et al*. 1995, Volis *et al*. 2002). Studies on growth under 'stressed' conditions can help in the understanding of functional trade-offs, selection pressures and evolutionary trends that may be obscured under optimal conditions (Pigliucci *et al*. 1995, Volis *et al*. 2002). Phenotypic plasticity can be conveyed graphically as a reaction norm to show the relationship between the two factors which affect the phenotype, environment (the 'stress') and genotype (the trait) (Pigliucci 2005, Kovnat 2007):



These reaction norms show that when the slope equals zero (i.e. is a flat line parallel to the environmental axis) there is no plasticity for that trait (graph A and B). If the slope is not equal to zero the genetic expression could be plastic (graph C and D). Plot D shows variation in the plastic response (genetic variation for plasticity); this could be subject to selection if the plastic expression of the genotype represents a genetic locus (Pigliucci 2005, Kovnat 2007). There could also be cases where the populations have no genetic variation (e.g. clonal organisms) but they still express a plastic response.

3.2.2 Choice of populations

Three populations were selected from the common garden experiment (Chapter 2) to be used in this plasticity study. The three populations were; Broken River lowland, Broken River forest and Broken River alpine. Each population represents a different habitat; **(1)** lowland close to the road, 750 m in altitude, **(2)** mid-forest, 1100 m, and **(3)** above the tree line in the alpine tussock grassland, 1450 m (refer to sites map Figure 1.4). Throughout this section these populations will be referred to as 'habitats' and will be labelled; 'lowland', 'forest', and 'alpine'.

3.2.3 Experimental design

Seed heads were collected from 15 randomly-chosen individuals (mother plants) from each of the three populations from the common garden. *Hieracium lepidulum* is an obligate apomict (Chapman *et al*. 2004) so the offspring of each mother plant can be considered genetically identical (Kultunow *et al*. 1995, Volis *et al*. 2002) and can be regarded as a single genotype. Between 15 and 20 seeds from each mother plant were sown onto filter paper in

Petri dishes. These were placed in a growth cabinet (16 hours – light, 25 °C; 8 hours – dark, 15 °C) to aid germination. Filter paper was kept damp using distilled water. After emergence, seedlings were transferred to 15-cm pots (initially 6 seedlings per pot) with standard soil mix. At the two-leaf stage (after approximately 2 weeks) three randomly-selected seedlings per mother plant were transferred to 10-cm pots with equal amounts of standard soil mix, a single plant per pot. Each of the three plants per genotype per population was subjected to one of the three treatments, giving a total of 45 individuals. Plants were set up in a random-blocking technique for each treatment.

The experiment was carried out in a glasshouse at the University of Canterbury, Christchurch. The three treatments were:

1) Control – Optimal levels of both factors, light and water.

2) Drought – Low levels of water and optimal levels of light.

3) Shade – Low levels of light and optimal levels of water.

Each treatment was set up on its own table in the glasshouse, as follows:

Control:

Plants in this treatment were grown in full available light (PAR = 200 $\mu molm^{-2}s^{-1}$). To determine the optimal water amount for this treatment I calculated the field capacity weight, the amount of water held in the soil after the excess water has drained away. A pot was filled with the equivalent amount of soil as used in the planted pots to be subjected to the three treatments. The weight of this pot was determined when the soil was completely dry (to be used in drought calculations) and then when it was at field capacity. To do this a large electronic balance accurate to 0.01 g was used. The field capacity weight was used as the

optimal water regime for the plants in the control treatment. On alternate days each plant in the control treatment was weighed and filled up with water to reach the field capacity weight.

Drought:

Plants in this treatment received the same light levels as in the control but only 50 % of the water. 50 % field capacity weight was calculated by subtracting the weight of a pot with dry soil from the weight at field capacity. This gives the weight of the water required to reach field capacity from dry soil and half of this weight gives 50 % field capacity weight. On alternate days each plant pot in the drought treatment was weighed and filled with water to the 50 % field capacity weight (modified method from Earl 2003).

Shade:

Plants in this treatment received the same water regime as in the control but only 10 % of the light (PAR = 20 $\mu molm^{-2}s^{-1}$). I determined the light level to be used as a shade 'stress' by measuring the amount of light that reached the ground through a stand of dense trees, replicating growth in dense forest. A large wooden frame was erected on top of the table and covered with green plastic gauze which only let through 10 % of the light available to the plants.

3.2.4 Phenotypic measurements

The same plant performance traits which were measured in the common garden (Chapter 2) were used for this experiment:

- maximum leaf length (mm)
- number of flowering stems
- number of buds
- number of inflorescences
- number of days until first flower
- number of seed heads
- number of seeds per seed head

The length of the longest leaf is commonly used as a relative measure of plant size (Wesselingh *et al*. 1997, Buckley *et al*. 2003). The other six performance traits give a measure of the reproductive output of each individual (Pigliucci *et al*. 1995, Miller 2005).

For analysis, the maximum value recorded for each individual over the entire study period was used for leaf length, number of flowering stems, buds and inflorescences. The sum of all seed heads produced throughout the study period for each individual was used for analysis. Fifteen seed heads were sampled from each individual and seeds counted to give a mean value for the number of seeds per seed head. Some individuals produced few or no seed heads in this study; for these individuals all the seed heads produced were used to determine the mean number of seeds per seed head. Each performance indicator was measured on a weekly basis for 20 weeks.

3.2.5 Analysis

Three types of statistical/graphical analyses were conducted: **(1)** analysis of variance (ANOVA) to determine genetic, environmental and interaction effects; **(2)** multiple comparison tests among the means of treatments and habitats; **(3)** reaction norm plots for each performance measure to visually compare patterns of plasticity and the interaction between treatment and habitat (treatment*habitat).

(1) ANOVA was used to assess the genetic, environmental and interaction effects. ANOVA was appropriate to test the overall treatment effects (phenotypic plasticity), the overall habitat effects (genetic variation among habitats/populations) and treatment*habitat interactions (genetic variation for plasticity). Both treatment and habitat were fixed effects. The null hypothesis for each factor and their interaction was that there were no statistically significant differences among them for the performance measure (phenotypic trait).

This model could not assess the habitat responses within a single treatment. These were analysed separately with an analysis of variance, where the genotypes within each habitat were regarded as replicates. This ANOVA was used to determine if there was any genetic variation among the habitats within each treatment; genetic variation for the plastic response to a specific treatment. The control treatment is essentially the equivalent of a common garden. The results in chapter two found that there is no genetic variation in these performance traits among the *H. lepidulum* populations in a controlled, optimal environment; therefore, we would expect to find no variation among the habitats in the control treatment. If the ANOVA shows a significant result within either the drought or shade treatments, it

indicates that the populations from different habitats vary in their plastic response; the pattern of genetic variation expressed is environment dependent.

Frequency distributions were determined for all performance measures. The statistical distributions were skewed for some of the count variables (number of flowering stems, number of buds, number of flowers, days until first flower, and total number of seed heads). This data required transformation, either $\log_e$ or square root, to satisfy the assumptions of the analysis (homogeneity of variance and normality). Initial size at planting did not vary among the individuals; therefore it did not need to be included as a covariate in the analyses as it will not have any influence on the final performance outcome. ANOVAs were then determined for all seven performance measures. All data analysis was done using R 2.6.0 (R Development Core Team 2007).

(2) Multiple comparisons of the means were carried out using the Tukey's Honest Significant Differences (Tukey's HSD) method to determine the statistical significance of overall differences among treatments. These results, by comparing each 'stress' treatment to the control, explain the amount of phenotypic plasticity (i.e. the degree of the plastic response). Tukey's HSD test applies a value of $\alpha = 0.05$ to the whole analysis (comparison of all the means) instead of for each single comparison. This sets the family-wise error rate, reducing the α value for each specific comparison, thus avoiding the problem of a Type I error occurring.

(3) Reaction norm graphs were plotted for each of the seven performance measures for the three habitats. The sequence in which the treatments appear on the environmental axis is *drought – control – shade*; this makes it visually easier to examine the direction and degree of departure of the plastic response from the control.

3.3 Results

The first ANOVA was to test the overall treatment effects (phenotypic plasticity), the overall habitat effects (genetic variation among habitats/populations) and treatment*habitat interactions (genetic variation for plasticity). Only four of the performance measures (number of buds and flowers, days until first flower and total number of seed heads produced) showed highly significant phenotypic plasticity in response to the treatments (Table 3.1). The ANOVA found no significant genetic variation among habitats for all performance traits except number of flowering stems. The number of flowering stems only exhibited slightly significant ($p = 0.031$) variation among habitats across the treatments. Since the common garden experiment (Chapter 2) established that there is no genetic variation in this trait among populations, I believe this slightly significant result is due to small sample sizes. There was no genetic variation for plasticity (treatment*habitat interaction) detected for any of the performance traits. The second analysis of variance was used to determine if there was any genetic variation among the habitats within each treatment (i.e. genetic variation for the plastic response to a specific treatment). For all performance measures there were no statistically significant differences detected among the habitat means within each treatment (Table 3.2). These results show that there is no variation among habitats in their plastic responses to drought and shade stress. They also confirm that there is no genetic variation for

these traits among populations, as there are no differences in habitat performance trait means within the control treatment.

Performance measure	Habitat		Treatment		Habitat*Treatment	
	F value	p value	F value	p value	F value	p value
Maximum leaf length	0.516	0.598	0.666	0.516	2.206	0.073
Number of flowering stems	3.562	0.031 *	1.957	0.146	0.349	0.844
Number of buds	1.947	0.147	38.28	$1.9e^{-13}$ ***	2.261	0.067
Number of inflorescences	1.050	0.353	90.60	$<2e^{-16}$ ***	1.103	0.359
Days until first flower	1.719	0.184	8.657	$3.2e^{-4}$ ***	0.661	0.620
Total number of seed heads	1.773	0.175	97.93	$<2e^{-16}$ ***	1.471	0.216
Seeds per seed head	3.070	0.1319	1.583	0.210	0.226	0.924

Table 3.1: Analysis of variance for differences in performance measures among habitats, among treatments, and the habitat*treatment interaction.

*** Highly significant ($p < 0.001$); ** Significant ($0.01 < p < 0.001$); * Slightly significant ($0.05 < p < 0.01$); ns, not significant.

Performance measure	Control		Drought		Shade	
	F value	p value	F value	p value	F value	P value
Maximum leaf length	1.421	0.254 ns	0.698	0.504 ns	3.052	0.059 ns
Number of flowering stems	2.365	0.108 ns	0.322	0.727 ns	1.885	0.166 ns
Number of buds	3.066	0.058 ns	1.660	0.204 ns	1.358	0.270 ns
Number of inflorescences	1.580	0.219 ns	0.965	0.386 ns	1.401	0.258 ns
Days until first flower	1.607	0.214 ns	1.626	0.210 ns	0.690	0.508 ns
Total number of seed heads	1.831	0.174 ns	1.100	0.343 ns	1.486	0.239 ns
Seeds per seed head	1.007	0.375 ns	2.242	0.120 ns	0.965	0.390 ns

Table 3.2: Analysis of variance for differences in performance measures among habitats within each treatment.

*** Highly significant ($p < 0.001$); ** Significant ($0.01 < p < 0.001$); * Slightly significant ($0.05 < p < 0.01$); ns, not significant.

Multiple comparisons of the means were carried out using Tukey's Honest Significant Differences (Tukey's HSD) tests to determine the statistical significance of overall differences among treatments for each performance trait. These results, by comparing each 'stress' treatment to the control, explain the amount of phenotypic plasticity (i.e. the degree of the plastic response). The Tukey's HSD plots show these results clearly; if the confidence interval line intersects zero, the treatment means are not significantly different. The comparisons of the treatment means for maximum leaf length, number of flowering stems and number of seeds per seed head showed no phenotypic plasticity across the treatments (Figure 3.1), confirming the ANOVA results. The reproductive traits; number of buds, number of inflorescences, days until first flower and total number of seed heads, all exhibited significant plasticity in response to the shade 'stress' but none to drought (Figure 3.2).

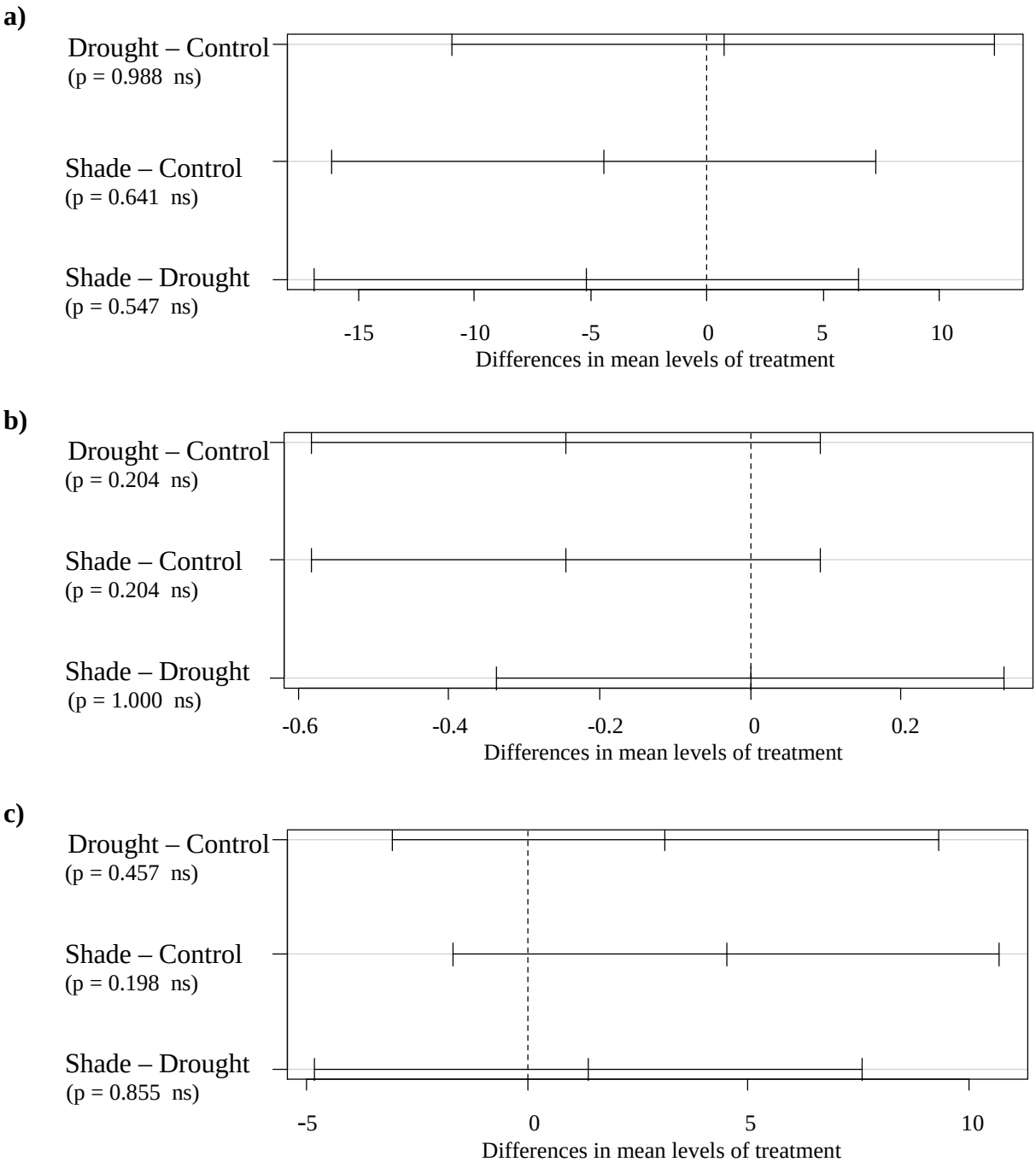
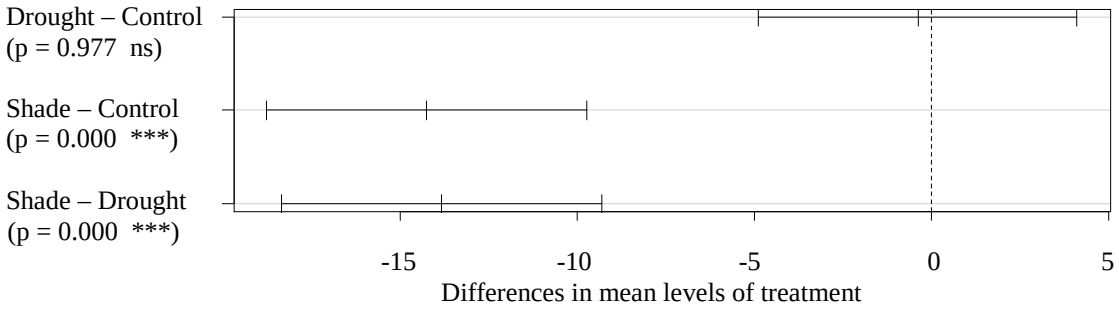


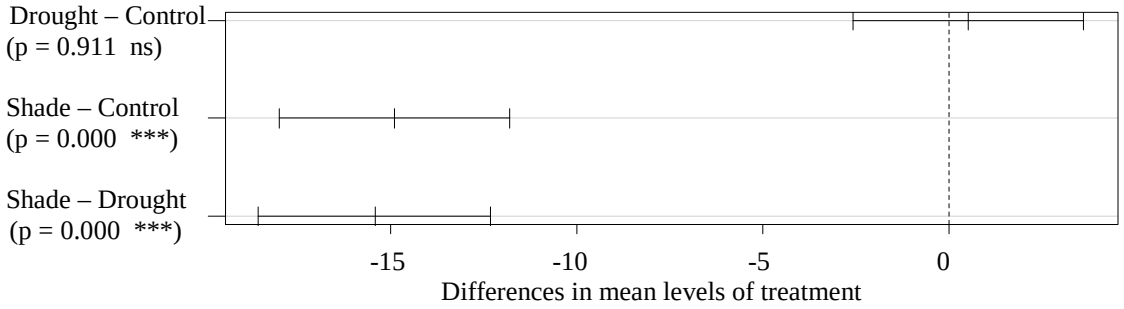
Figure 3.1**:** 95 % family-wise confidence level plot comparing the performance trait means among treatments across the three populations for; (a) maximum leaf length, (b) number of flowering stems, and (c) seeds per seed head. p values included to show significance levels:

*** Highly significant ($p < 0.001$); ** Significant ($0.01 < p < 0.001$); * Slightly significant ($0.05 < p < 0.01$); ns, not significant

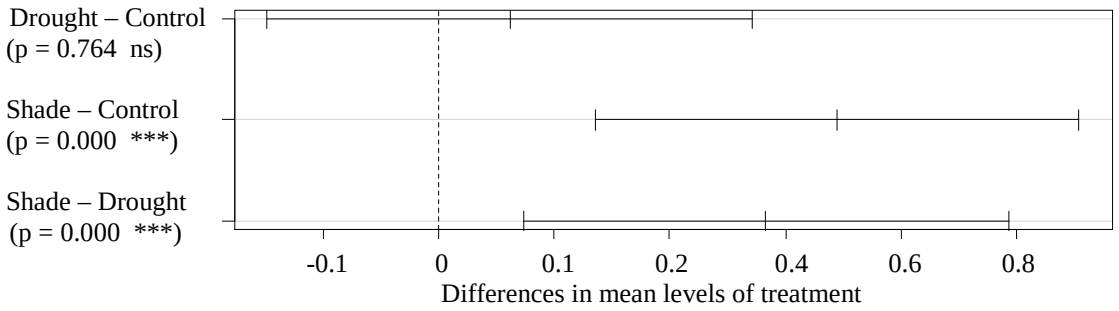
a)



b)



c)



d)

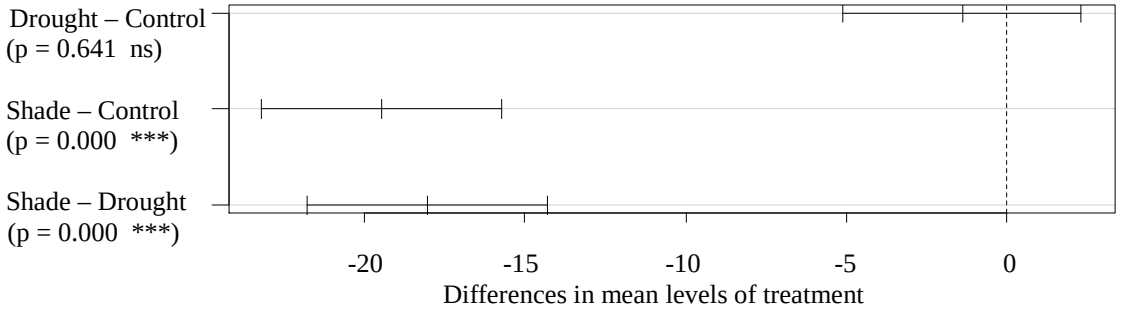


Figure 3.2**:** 95 % family-wise confidence level plot comparing the performance trait means among treatments across the three populations for; (a) number of buds, (b) number of inflorescences, (c) days until first flower, and (d) total number of seed heads. p values included to show significance levels:
*** Highly significant ($p < 0.001$); ** Significant ($0.01 < p < 0.001$); * Slightly significant ($0.05 < p < 0.01$); ns, not significant

The reaction norm plots (Figure 3.3) provide a more detailed illustration of the across-treatment trends of the populations. The reaction norms show the following patterns of plasticity for each performance trait:

(1) Maximum leaf length (Figure 3.3 a). Trend lines for all habitats are almost parallel to the treatment axis; they show low plasticity. There is also no observable genetic variation among habitats as the trend lines are all clustered together. The 'stress' treatments did not affect this performance trait.

(2) Number of flowering stems (Figure 3.3 b). Trend lines for all habitats tend to be parallel to the treatment axis; they show low plasticity. The three habitat trend lines are slightly separated at the control and shade end of the environmental axis suggesting there is genetic variation for this trait among the populations. The common garden experiment found no genetic variation in this performance trait among populations and the ANOVA results did not find significant variation among habitat means within the control or shade treatments. I believe the small separation among the trend lines is a reflection of the small population sizes.

(3) Number of buds (Figure 3.3 c). All habitats show low plasticity in response to drought 'stress'; the trend lines tend to be parallel to the environmental axis between the control and drought treatments. All three populations exhibit a very pronounced plastic response to shade 'stress'; plants raised under shade produced significantly fewer buds. There was no variation among the habitats in their plastic responses to the treatments for this trait.

(4) Number of inflorescences (Figure 3.3 d). This performance trait responded to the treatments in the same direction as for number of buds. Populations showed low plasticity in response to drought but a significant response to shade, with plants producing considerably fewer flowers when raised under shade 'stress'. There was no variation among the habitats in their plastic responses to the treatments for the number of inflorescences.

(5) Days until first flower (Figure 3.3 e). This performance trait displayed low plasticity to drought. Each habitat showed comparable plastic responses to shade 'stress' by significantly delaying the onset of flowering time.

(6) Total number of seed heads (Figure 3.3 f). The three populations exhibited the same responses to the treatments as for the previous three traits. Low plasticity in response to drought, and a significant plastic response to shade with the total number of seed heads greatly diminished.

(7) Seeds per seed head (Figure 3.3 g). Populations display flat reaction norms illustrating low plasticity in response to the treatments for this performance trait. The habitat trend lines were also clustered together, indicating no genetic variation among the populations for the number of seeds produced per seed head.

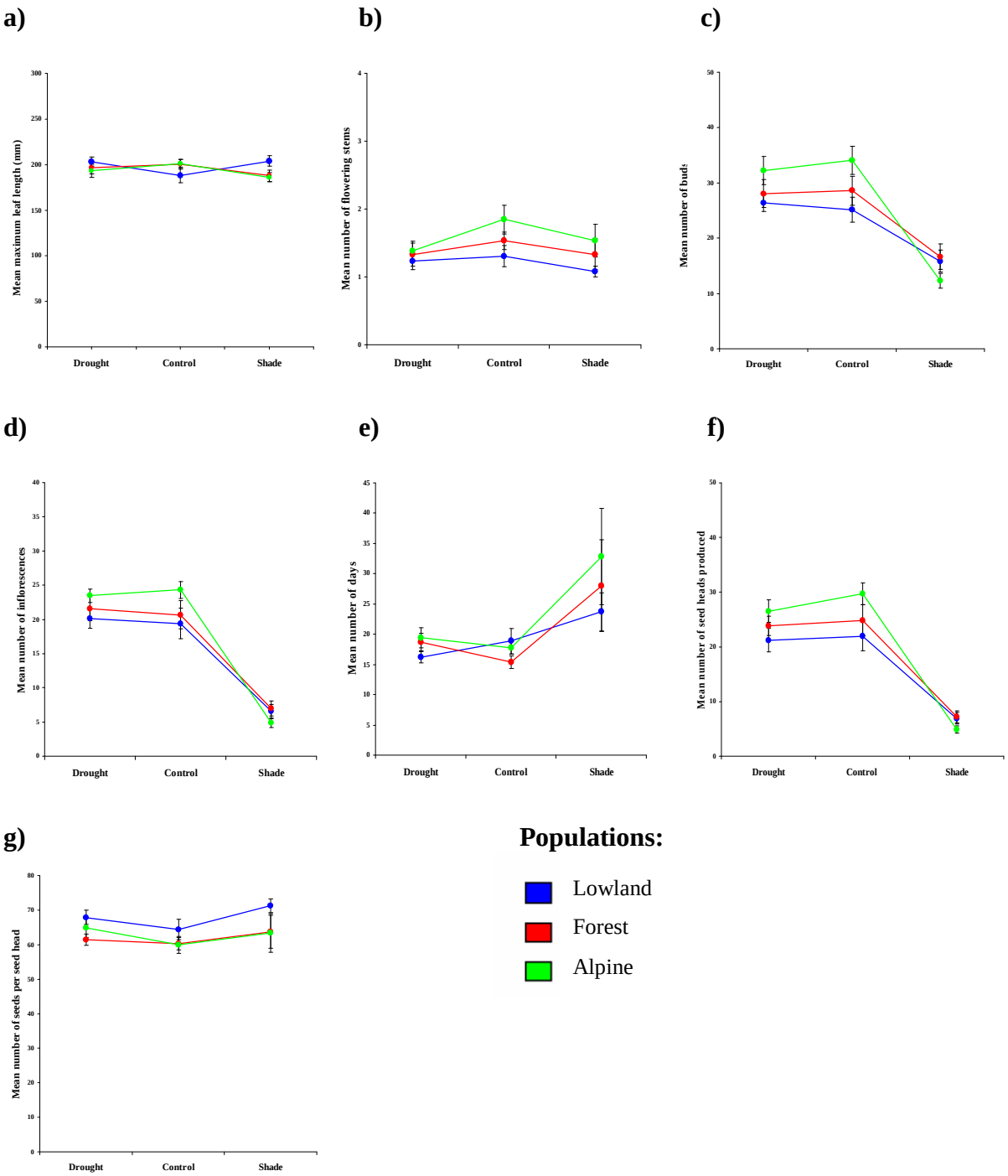


Figure 3.3: Reaction norms for three populations (representing three habitats; lowland, forest, and alpine) of *Hieracium lepidulum* to a set of treatments (drought, control and shade) for the phenotypic measures; (a) maximum leaf length (mm), (b) number of flowering stems, (c) number of buds, (d) number of inflorescences, (e) days until first flower, (f) total number of seed heads produced, and (g) seeds per seed head. Vertical bars denote the standard error of the mean.

3.4 Discussion

The three populations used in this study, which originated from three different habitats ('lowland', 'forest' and 'alpine'), did not show genetic variation for the observed traits among the populations. This was evident for each performance trait as the habitat means did not differ significantly within the control treatment. This confirms the results of the common garden experiment (Chapter 2); the phenotypic variation in these performance traits, which is visible among different habitats in the field, is best explained as the result of phenotypic plasticity.

The two 'stress' treatments, drought and shade, used in this experiment induced very different plastic responses among the performance traits. For all the seven traits studied, none showed any plasticity in response to the drought treatment. Habitat trait means did not significantly differ between the drought and control treatments, suggesting that drought in fact is not a 'stress' for *H. lepidulum.* To determine the degree of drought tolerance in this species, similar plasticity studies need to be conducted across a water gradient. From the perspective of the invasive success of this species, drought tolerance would confer a great advantage. Individuals would not only have the ability to successfully invade novel, arid environments, but would also be able to thrive in existing habitats when faced with a drought. As the reproductive traits were not affected by drought 'stress' it potentially could allow populations to spread into areas occupied by plants which cannot tolerate drought conditions.

In response to the shade treatment, four of the reproductive traits elicited a significant plastic response. For each habitat the number of buds, flowers and seed heads produced were considerably fewer than in the control treatment and there was also a substantial delay in flowering time. There was no variation in the plastic response among the habitats for these four traits (i.e. no genetic variation for plasticity). If there is no genetic variation for plasticity, there is no variation on which selection can act, suggesting that adaptive phenotypic plasticity may not occur in populations of *H. lepidulum.* Populations did not show plasticity in response to shade for maximum leaf length or number of flowering stems; the trait means for each habitat were not significantly different from the control. This implies that plants reduced allocation to flowering and producing seed heads and concentrated the allocation of their resources on producing large leaves and more stems. The lack of response of these two phenotypic traits to shade 'stress' has been found in several studies on herbaceous and woody species (Navas and Garnier and refs. within 2002). Maintaining the normal number of stems may enhance competitive ability in light-limiting habitats by spreading their leaves in space, and large leaf sizes will aid in the capture of available light (Meekins and McCarthy 2000, Navas and Garnier 2002, McAlpine and Jesson 2007, Bell and Galloway 2008). These 'shade avoidance' tactics result in an increase of fitness at the level of the individual, by enhancing light capture, but the trade-off between reproductive effort and above-ground biomass results in a reduction of fitness at the population level, due to decreased reproduction (Meekins and McCarthy 2000, Bell and Galloway 2008). The results suggest that *H. lepidulum* is shade-intolerant, which is supported by the natural distribution of this species; abundance is very low in closed-canopy forest (Miller 2005, pers. obs.). Invasion of *H. lepidulum* into closed-canopy forest habitats is limited by the low reproductive

output in low light conditions. Since populations scarcely occupy shaded habitats, the ability to maintain large leaf sizes and normal number of stems when raised under shade 'stress' has probably developed in nature as a response to avoid shading from neighbouring competitors. *Hieracium lepidulum* populations can be found flourishing in forest canopy gap, creek and edge habitats (Miller 2005, pers. obs.), which suggests that *H. lepidulum* can tolerate shade (from neighbouring trees) up to a certain degree. The degree of shade-tolerance could be determined in further plasticity studies using a light gradient.

3.5 Summary

The aim of this section was to investigate the genetic variation, phenotypic plasticity and genetic variability for plasticity in populations of *Hieracium lepidulum* subjected to two types of abiotic 'stress', shade and drought (compared to an 'optimal' control). The populations came from three different habitats, 'lowland', 'forest' and 'alpine' within the Broken River catchment. For the seven performance traits studied, only four of the reproductive traits showed significant plasticity in response to the treatments. The phenotypes produced in the shade treatment were very distinct compared to the drought 'stress' and the control. The drought treatment did not induce any form of plastic response compared to the control for any phenotypic trait studied, suggesting that *Hieracium lepidulum* populations are drought tolerant. The shade treatment induced a significant reduction in the number of buds, flowers and seed heads produced and a considerable delay in flowering time. The results suggest that *H. lepidulum* is shade intolerant, which is evident in its scarcity in nature in closed-canopy forest habitats.

Chapter 4

4. Genetic diversity and population genetic structure

4.1 Introduction

Understanding of genetic diversity and population genetic structure is necessary for evolutionary studies of mating systems and relatedness; it is also a requirement for successful weed management programmes (Jasieniuk and Maxwell 2001, Sun *et al*. 2001, Hufbauer 2004). Population genetics can help us comprehend the biology of invasions, which will aid in weed control by revealing which species are present, the invasion origin and the methods and distance of migration (Jasieniuk and Maxwell 2001, Walker *et al*. 2003, Hufbauer 2004). Population genetics concentrates on determining the amount and structure of genetic variation within and among populations, leading to the understanding of the evolutionary processes occurring (Hufbauer 2004).

Hieracium lepidulum is a seriously invasive weed in New Zealand. Over the last 50 years the frequency and abundance of *H. lepidulum* has been progressively increasing throughout the South Island of New Zealand. As this species is capable of invading and dominating in such a wide range of habitats, it poses a serious threat to indigenous plant communities (Wiser *et al*. 1998, Chapman *et al*. 2004). Understanding of the evolutionary colonisation processes of *H. lepidulum*, through the use of population genetics, will aid in the development of management programmes to control and eventually eradicate this destructive invader.

H. lepidulum is a diplosporous, obligate apomict; it reproduces through the production of clonal seed (Chapman *et al*. 2004). This type of asexual reproduction through seed is a breeding system which does not involve meiosis or fertilisation, and is essentially a complete transmission of the entire maternal genotype to the offspring (Asker and Jerling 1992, Kultunow *et al*. 1995, Chapman *et al*. 2004). Apomixis fixes a particular genotype, and apomicts are therefore described as clonal lineages (Asker and Jerling 1992, Storchova *et al*. 2002, Chapman *et al*. 2004). This mechanism of reproduction results in a colossal number of asexual clones, and theoretically the populations should harbour very low levels of genetic variation, with the exception of possible somatic mutations (Bayer *et al.* 1990, Storchova *et al*. 2002, Chapman *et al*. 2004, Arnaud-Haond *et al*. 2007). Low levels of genetic variation are typically associated with evolutionary dead ends, because natural selection has very little variation on which to act (Fox *et al*. 1996). There have been no recordings of sexual diploids in *H. lepidulum*, so intraspecific hybridisation as a means of providing genetic variation is very unlikely (Chapman *et al*. 2004). However, previous work on New Zealand populations of *H. lepidulum,* using intersimple sequence repeats (ISSRs) (Chapman *et al.* 2004) and allozymes (pers. obs. final-year BSc project 2005), have found there to be low levels of genetic variation within populations, and high levels of variation among populations.

4.1.1 Objective three

The first part of this section is dedicated to a review of some of the various techniques and molecular markers utilised in population genetics. I will briefly explain the techniques involved for each approach and then outline the advantages and limitations in their use. The aim of the second part of this section is to determine the genetic diversity and population

genetic structure of *Hieracium lepidulum* populations in the Craigieburn Range. Analysing the genetic variation within and among these populations will determine if this species is genetically identical as the breeding system dictates, or if in fact there is high levels of genetic variation potentially resulting from recombination and outcrossing (Storchova *et al*. 2002, Chapman *et al*. 2004, Arnaud-Haond *et al*. 2007). Understanding the population genetic structure of *Hieracium lepidulum* will help uncover the evolutionary colonisation processes which have occurred during its invasion. Population genetics can also determine: **(1)** clonal diversity, the number of distinct lineages, **(2)** clonal richness, the number of different lineages in the sample, and **(3)** clonal evenness, the distribution of individuals sampled among the different lineages (Arnaud-Haond *et al*. 2007).

4.2 Molecular technologies and their role in population genetics

The last 50 years has seen a great advancement in the techniques used in population genetics. Beginning with a time when there were only a handful of analytical methods, limited in their application and study organisms, to the present where any information required can be determined using a suite of techniques on any organism (Bachmann 2001). The development of the polymerase chain reaction (PCR) for amplifying specific stretches of DNA has led to major advances in molecular technologies applicable in population genetics, the most important being: the application of evolutionarily conserved PCR primers, the discovery of hypervariable microsatellite loci and DNA sequencing (Karp *et al*. 1996, Sunnucks 2000). Choosing the most appropriate technique not only depends on the specific question being asked but also **(1)** the extent of genetic polymorphism required to best answer the question, **(2)** the analytical or statistical approaches available for the technique and **(3)** the practicality

of time and costs of the materials (Parker *et al*. 1998, Sunnucks 2000). In this section I will briefly explain some of the various techniques available for population genetic studies, in order of increasing resolution, and their advantages and limitations in relation to the traditional questions asked in population biology.

4.2.1 Protein markers

Allozymes

Allozymes are distinct forms of a nuclear-encoded enzyme, encoded by different alleles at a single locus (Parker *et al*. 1998, Jasieniuk and Maxwell 2001). The late 1960s saw the first use of protein electrophoresis to determine population genetic variation among enzyme loci; this was achieved as individuals were able to be identified as homozygotes or heterozygotes at a particular locus (Parker *et al*. 1998, Jasieniuk and Maxwell 2001). Over the years allozymes have been used extensively in population genetics, with many studies of plant populations finding high levels of genetic diversity (Parker *et al*. 1998, Ouborg *et al*. 1999, Jasieniuk and Maxwell 2001).

Allozymes are relatively inexpensive and simple to use which makes them a popular technique in population studies (Jasieniuk and Maxwell 2001). The following allozyme procedure is a modified method from Parker *et al*. (1998). Prior to electrophoresis, proteins must be extracted from tissue samples. Samples are ground, usually in liquid nitrogen to prevent enzymes denaturing and losing their activity, then homogenised in specific buffers to extract the enzymes. Samples are applied to a starch or polyacrylamide gel to be separated by size, shape and/or charge along an electrical gradient. After electrophoresis, each gel is

stained for a specific enzyme which allows the visualisation (as coloured bands) of the positions of different allozymes. There must be a comprehensive understanding of the genetic basis of allelic variation for each enzyme to correctly analyse the gels; at a given locus, homozygotes usually produce one band, whereas heterozygotes typically yield two, three or five bands depending on the quaternary structure of the enzyme (Parker *et al*. 1998).

There are several advantages in using allozymes in population genetic studies. They are simple and cheap to use and large numbers of samples can be processed efficiently (Parker *et al*. 1998, Jasieniuk and Maxwell 2001). Allozyme data can be directly compared among studies and the techniques can be easily transferred to new taxa (Parker *et al*. 1998, Sunnucks 2000). The majority of allozymes are expressed as codominant Mendelian loci, allowing homozygous and heterozygous genotypes to be determined (Parker *et al*. 1998, Ouborg *et al*. 1999, Sunnucks 2000, Jasieniuk and Maxwell 2001). Allozymes can be used to elucidate heterozygosity, gene diversity, genetic differentiation and population genetic structure (Jasieniuk and Maxwell 2001).

Despite the advantages of using allozymes for population genetic studies they also have some major limitations. The genes encoding allozymes only represent a small portion of the entire genome (Jasieniuk and Maxwell 2001). Many allozyme studies require at least 10–20 independently segregating polymorphic loci to provide minimal statistical confidence (Parker *et al*. 1998). This is problematic as the overall variability of allozymes is relatively low (Sunnucks 2000). Some species are monomorphic for most allozymes and among taxonomic groups the amount of allozyme variation they possess is immensely different; on average

across taxa, polymorphic loci make up less than half of all loci and loci possessing more than three alleles are rare (Parker *et al*. 1998). Allozymes are not suitable genetic markers in many species due to the lack of sufficiently polymorphic loci needed to provide a statistically sound analysis. Another disadvantage associated with allozymes is that they may differ in their metabolic function and therefore be exposed to natural selection (Parker *et al*. 1998, Ouborg *et al*. 1999, Jasieniuk and Maxwell 2001). Many statistical models used in population genetic analysis assume that phenotypic differences among allozymes are selectively neutral (Jasieniuk and Maxwell 2001). Studies have found that selection can act on allozymes and on traits which they are genetically linked to; noncoding DNA rather than a gene product which is exposed to selection would be more ideal as a genetic marker (Parker *et al*. 1998). Allozymes are also unable to detect very small genetic differences; only nucleotide substitutions which change the net charge and therefore the mobility on the gel will be resolved (Jasieniuk and Maxwell 2001). In addition, some alleles may not be identified due to redundancy in the genetic code and similar migration distances on a gel (Jasieniuk and Maxwell 2001).

4.2.2 DNA markers

The development of molecular techniques has allowed population biologists to study variation of nucleic acid sequences (Parker *et al*. 1998, Jasieniuk and Maxwell 2001). Working with DNA and DNA markers has several advantages over allozymes; **(1)** the markers are selectively neutral, **(2)** markers can detect fine-scale genetic variation, **(3)** DNA is found in nearly all cells of all organisms, **(4)** DNA can be extracted from living and dead tissue, and **(5)** only very small amounts (e.g. nanograms) of DNA are needed as it can be

amplified with PCR (Parker *et al*. 1998, Ouborg *et al*. 1999, Jasieniuk and Maxwell 2001, Hufbauer 2004). Below I will discuss the commonly used molecular DNA techniques and their applications to studies of population genetics.

Restriction fragment length polymorphisms (RFLPs)

Restriction fragment length polymorphisms (RFLPs) were the first DNA markers to be used in studies of population genetics (Parker *et al*. 1998, Jasieniuk and Maxwell 2001). DNA is digested with restriction enzymes and the resulting fragments are separated by molecular weight with gel electrophoresis (Jasieniuk and Maxwell 2001). The fragments are then transferred to a filter by Southern blotting and the fragments containing sequences of interest are identified by hybridisation to labelled probes (Jasieniuk and Maxwell 2001). Genetic variation is determined by differences in restriction patterns among individuals or species, which can arise due to mutations which alter the restriction sites, or from insertions, deletions or sequence rearrangements between restriction sites (Karp *et al*. 1996, Jasieniuk and Maxwell 2001).

RFLPs are highly reproducible and can assess genetic variation within and among populations if there are sufficient polymorphic loci (Parker *et al*. 1998). RFLPs are codominant which allows homozygous and heterozygous genotypes to be distinguished (Karp *et al*. 1996, Sunnucks 2000, Jasieniuk and Maxwell 2001). This technique can use nuclear or organelle DNA, mitochondrial or chloroplast (Sunnucks 2000). Organelle DNA RFLPs are useful for assessing population genetic divergence over large geographic areas (Jasieniuk and Maxwell 2001). Organelle RFLPs usually elucidate greater population

differentiation than nuclear-encoded markers because; **(1)** organelle DNA is inherited as a single unit and is not subject to recombination, **(2)** nuclear genes are dispersed by seeds and pollen, whereas organelle DNA only by seeds, resulting in gene flow usually being lower for organelle DNA (Sunnucks 2000, Jasieniuk and Maxwell 2001).

RFLPs do have their limitations. Southern blotting and hybridisation is very time-consuming and expensive for the quantity of information gathered (Jasieniuk and Maxwell 2001). A good supply of probes is also needed and if heterologous probes are not available, new cDNA or genomic probes must be developed (Karp *et al*. 1996). The greatest limitation of this technique is that it requires relatively large quantities (e.g. 10 μg per digestion) of high-quality DNA, rendering RFLPs inadequate with limited amounts of source material or preserved tissue (Karp *et al*. 1996, Jasieniuk and Maxwell 2001).

Randomly amplified polymorphic DNAs (RAPDs)

The development of PCR for amplifying DNA led to an array of new molecular technologies that overcame many of the limitations of RFLPs (Karp *et al*. 1996, Jasieniuk and Maxwell 2001). RAPD markers are produced by PCR amplification of random DNA sequences with short oligonucleotide primers, usually ten base pairs long, of an arbitrary sequence (Parker *et al*. 1998, Jasieniuk and Maxwell 2001). RAPD analysis uses a single primer of randomly chosen sequence; this is a contrast to standard PCR, which uses two different primers whose base composition is determined by a known sequence of the fragment to be amplified (Jasieniuk and Maxwell 2001). Amplified fragments are those regions of the genome which are flanked by ‘inward-oriented’ sequences complimentary to the primer (Parker *et al*. 1998).

Amplification products are separated on agarose gels in the presence of ethidium bromide and visualised under ultraviolet light; allelic variation is determined by the presence or absence of amplification products (Karp *et al*. 1996, Parker *et al*. 1998).

The RAPD technique has several advantages over allozymes and RFPL; **(1)** Rapid examination of genetic variation at many loci from different regions of the genome, **(2)** only small amounts of DNA are needed (10 ng per reaction), **(3)** no prior knowledge of the DNA sequence is needed to produce primers, **(4)** there are many different commercially available primers which can be used among taxa, **(5)** they are easy and fast to use and less expensive than RFLPs, and **(6)** RAPD markers have high variability (Karp *et al*. 1996, Parker *et al*. 1998, Jasieniuk and Maxwell 2001). RAPDs have been used to differentiate individuals, cultivars, accessions and populations (Karp *et al*. 1996, Jasieniuk and Maxwell 2001).

RAPDs do also have limitations. As RAPD primers are very short in length, they may produce some artefact amplification products, and template DNA quality and amplification conditions must be strictly controlled to ensure reproducible banding patterns (Karp *et al*. 1996, Parker *et al*. 1998, Jasieniuk and Maxwell 2001). RAPD markers are also dominant; amplification products are scored as present or absent and heterozygotes can not be determined (Karp *et al*. 1996, Parker *et al*. 1998). The loss of a priming site results in an absence of the enclosed amplified segment, not simply seen as a shift of mobility on a gel; therefore heterozygotes may only appear as differences in band intensity which is not reliable for analysis (Parker *et al*. 1998). Consequently, parental origins of alleles may be unattainable for RAPD markers (Parker *et al*. 1998). Without pedigree analysis, it is

impossible to determine the identity (assign markers to specific loci) of multi-band profiles (Karp *et al*. 1996, Jasieniuk and Maxwell 2001). An enormous assumption is made when analysing RAPDs; the presence of a band of apparently identical molecular weight in different individuals signifies that the two individuals share the same homologous fragment (Karp *et al*. 1996). This cannot be taken as fact, especially considering single bands can sometimes be comprised of several co-migrating amplification products (Karp *et al*. 1996, Jasieniuk and Maxwell 2001).

Variable number of tandem repeats (VNTRs)

A powerful technique for studying genetic diversity involves examining the hypervariable regions of the genome comprising tandemly repeated simple sequences (Jarne and Lagoda 1996, Karp *et al*. 1996, Jasieniuk and Maxwell 2001). The repeats vary in number (and therefore length) between individuals; hence their name 'variable number of tandem repeats' (Karp *et al*. 1996, Jasieniuk and Maxwell 2001). There are two main types of VNTR markers; **(1)** microsatellites (also called simple sequence repeats, SSR), which is where the basic repeat unit is between two and ten base pairs in length, and **(2)** minisatellites, where the basic repeat unit is longer (Jarne and Lagoda 1996, Karp *et al*. 1996, Parker *et al*. 1998, Jasieniuk and Maxwell 2001). VNTRs are widely dispersed through the plant genome (Karp *et al*. 1996, Jasieniuk and Maxwell 2001). This discussion will only involve microsatellite markers as they are the most popular in population genetic studies (Jasieniuk and Maxwell 2001).

Microsatellite analysis involves the development of PCR primers for the regions flanking a microsatellite repeat; the target region is then amplified using PCR, followed by high-resolution electrophoresis to allow separation of the microsatellite PCR products (Parker *et al*. 1998, Jasieniuk and Maxwell 2001). Fluorescently-labelled primers are commonly used and the amplified products can be separated on a sequencing gel in an automated sequencer. Polymorphisms are detected by differences in the length of the amplified product; allele size can be scored using 'genotyping' software.

Microsatellites are the most powerful molecular markers for several reasons: **(1)** high resolution. Allelic variation can be resolved to differences in size of as few as two base pairs (Parker *et al*. 1998, Jasieniuk and Maxwell 2001); **(2)** high mutation rates and variability. High variability within loci can reduce the total number of loci required to be screened in order to distinguish genotypes. Mutation rate is very important as it is a major determinant of variability within populations; microsatellites have a mutation rate in the order of 10^{-5}–10^{-2} (Jarne and Lagoda 1996, Procaccini and Mazzella 1998). Due to the high mutation rates there can be as many as 30–50 allelic variants detected at a single locus (Karp *et al*. 1996, Parker *et al*. 1998, Jasieniuk and Maxwell 2001); and **(3)** codominance. The codominant nature of microsatellites allows homozygotes and heterozygotes to be clearly distinguished (Jarne and Lagoda 1996, Jasieniuk and Maxwell 2001). These attributes of microsatellites make them extremely useful for population genetic studies; they are sensitive enough to determine individual genotypic identity and parentage (Sunnucks 2000, Jasieniuk and Maxwell 2001).

The major limitation for microsatellites is the identification and development of the PCR primers, involving cloning and sequencing which is very time-consuming (Parker *et al*. 1998, Jasieniuk and Maxwell 2001). This disadvantage is worsened as it is thought that microsatellite primers do not amplify the same locus across taxa, unless the microsatellite region where the priming sites are located is flanked by highly conserved sequences (Parker *et al*. 1998). Although, recent studies have found that microsatellite primers can be used among closely-related taxa; this allows scientists to first try the already-available primers to possibly avoid the time-consuming, expensive primer development process (Parker *et al*. 1998, Jasieniuk and Maxwell 2001, Jump *et al*. 2002, Andrew *et al*. 2003, Hale *et al*. 2005, Lemes *et al*. 2007, Panova *et al*. 2008, Xu *et al*. 2008). Microsatellites are now more widely used, and therefore the number of available primers that will amplify across taxa will increase.

4.2.3 Summary

There are several attributes which are favourable in a molecular marker:

1) Assayable by PCR; this allows the use of DNA of low quantity and quality and can also target specific DNA regions.

2) Comparability; PCR primers which can amplify across a wide taxonomic range, thus allowing direct comparisons among studies.

3) DNA rather than protein; DNA can be extracted from ancient material, collection and storage of samples is easier, it is more variable and can be used for PCR.

4) Gene genealogies; molecular genealogies can describe evolutionary processes, and markers which give allele frequency and sequence data can be useful for this.

5) Many separate loci available; being able to use multiple markers increases the sensitivity.

6) Rapid development and screening.

7) High overall variability.

8) Single-locus as opposed to multilocus markers; multilocus techniques (examining several genes simultaneously) are imprecise and have great technical and analytical disadvantages, such as dominance; data is also of limited comparability among studies. Single-locus markers are codominant and they provide reliable data, which is comparable among studies, for input into precise analyses (Sunnucks 2000).

On the basis of these attributes, microsatellites are the most favourable markers in population biology (Jarne and Lagoda 1996, Parker *et al*. 1998, Sunnucks 2000, Jasieniuk and Maxwell 2001). Their lack of ability at being able to amplify across taxa will be reduced with the increase in development of primers which work well within a family or order (Parker *et al*. 1998).

4.3 Methods

4.3.1 Experimental background

Analysing the genetic variation within and among populations of *Hieracium lepidulum* from the Craigieburn Range will determine if this species is genetically identical as the breeding system dictates, or if in fact there is high levels of genetic variation potentially resulting from recombination and outcrossing (Storchova *et al*. 2002, Chapman *et al*. 2004, Arnaud-Haond *et al*. 2007). Understanding the population genetic structure of *Hieracium lepidulum* will help uncover the evolutionary colonisation processes which have occurred during its invasion. Population genetics can also determine; **(1)** clonal diversity, the number of distinct lineages,

(2) clonal richness, the number of different lineages in the sample, and **(3)** clonal evenness, the distribution of individuals sampled among the different lineages (Arnaud-Haond *et al*. 2007).

Microsatellite markers were used in this study to determine the genetic variation within and among populations, and the population genetic structure. Microsatellite markers are highly variable and are the most powerful markers to assess clonal diversity and membership; this is evident by the significant rise in the use of microsatellites for clonal studies (Arnaud-Haond *et al*. 2007).

4.3.2 Choice of populations

The populations chosen to use for this genetic analysis are those which were used in the common garden (Chapter 2). Populations of *H. lepidulum* were sampled from three geographically-separate catchment locations within Craigieburn Forest Park; Mount Cheeseman Skifield, Broken River Skifield, and Craigieburn Skifield basins. Within each location, populations were sampled from three different altitudes, which define three different habitats; **(1)** lowland close to the road, 750 m in altitude, **(2)** mid-forest, 1100 m, and **(3)** above the tree line in the alpine tussock grassland, 1450 m (refer to sites map Figure 1.4). This gave three replicates of each habitat, where each replicate is from one of the three geographically-separate locations. A total of nine populations of *H. lepidulum* were used for the genetic analysis:

- Broken River – lowland, forest and alpine
- Cheeseman – lowland, forest and alpine

- Craigieburn – lowland, forest and alpine

From each of the nine populations, 16 individuals were randomly selected to undergo genetic analysis; this gave a total of 144 individuals.

4.3.3 DNA extraction

DNA was extracted using a modified CTAB (hexadecyltrimethylammonium bromide) method from Weising *et al*. (1995). The following protocol was used to extract DNA from each of the 144 individuals. Approximately 1 cm^2 of a fresh young leaf was ground in a 1.5-ml tube using a micropestle with 800 µl of 2x CTAB buffer (0.1 M Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA (pH 8.0), 2 % CTAB) and 4 µl of 5 % β-mercaptoethanol, and then incubated at 60 °C for 30 minutes. After washing with 600 µl of 24:1 chloroform:isoamylalcohol and centrifuging for 5 minutes, 13 000 r.p.m. at 10 °C, the aqueous layer was transferred to a new 1.5-ml tube. This washing step was repeated; the resulting aqueous phase had 4 µl of 10 mg/ml RNase a (Fermentas) added and was incubated at 37 °C for 30 minutes to degrade the single-stranded RNA. DNA was precipitated by adding 500 µl of cold isopropanol and the tube was held at -20 °C for 30 minutes. The DNA was pelleted by; centrifuging at 13 000 r.p.m. for 10 minutes, the supernatant removed and the DNA pellet washed with 300 µl of 70 % ethanol, centrifuged at 13 000 r.p.m. for 10 minutes, and finally air-dried for 15 minutes. The pellet was resuspended in 100 µl of TE buffer (tris acetate; 10 mM Tris-HCl (pH 8.0), 1 mM EDTA). For each sample, 5 µl of the DNA extract mixed with 5 µl of 5x DNA loading buffer (BIOLINE) was electrophoretically separated on a 1.4 % agarose gel stained with ethidium bromide in 1x TAE buffer. Gels were

examined under ultraviolet light to visualise the quantity and quality of the DNA extracts. DNA extracts were stored at -20 °C.

4.3.4 Microsatellite markers

Zini and Komjanc (2007) recently developed nine microsatellite marker loci in *Hieracium pilosella,* a close relative of this study species, *Hieracium lepidulum.* The markers were highly variable; the analysis of loci yielded 5–33 alleles per locus, with individuals possessing between one and six alleles per locus. As microsatellite primers can be used among closely-related taxa (Parker *et al*. 1998), I chose to use the primers designed for *Hieracium pilosella* to avoid the time-consuming and costly process of primer development. The nine microsatellite primer pairs (Table 4.1) were ordered from Invitrogen; these were used for initial testing for amplification in *H. lepidulum*.

Locus name	Repeat motif	Primer sequence (5'–3')	Ta	Allele size range	Na	Ni
HP-10	$(CAA)_7$	CGGACAGGAATTACCGAGAG GCACATGAGCTTCATTTCCTT	63	159–243	5	1–5
HP-26	$(GTT)_{14}$	TGGGTCGATTATTGGGATTG GCACACTTGTTCCCACCAA	64	143–161	6	1–4
HP-42	$(CAA)_9$	ACCGAGCCGGGTCACTA TTCCCATGAGAACTGCTGAA	60	129–168	10	2–5
HP-12	$(GTT)_{10}$	CCCCTGGAGATGTGAGTTGT TTCCATTCCACCAAGGAGAC	64	115–271	11	1–5
HP-34	$(CAA)_9$	CGATCCTTCCTTCACTCCAA ATTGCCCTTGATGAGTCCTG	64	132–231	15	1–4
HP-9	$(GA)_{22}(GT)_{11}$	TCTCTTCCTTCCATTCTCATTTG TCACGTCATGCTCCAATCTC	63	147–217	28	1–4
HP-3B	$(GT)_{16}$	CCCCAAAACTCCCAATACAT TCATTGGGACTTCCACAAGTT	62	115–157	17	2–6
HP-26B	$(GA)_{20}$	TCCATGAGACGATGTTGGAA TCACACACACACACACACACA	64	142–210	24	1–4
HP-87	$(GA)_{25}$	CAGTTTCAGTCGAGTTTCATACG TCGTTCCACTGTTTGAGTCG	63	132–202	33	1–4

Table 4.1: The nine polymorphic microsatellite loci isolated in *Hieracium pilosella*.

(Table modified from Zini and Komjanc 2007). Ta – annealing temperature, Na – total number of alleles, and Ni – number of alleles per individuals.

4.3.5 Initial primer testing

This initial testing was to determine if the primers designed for *Hieracium pilosella* would also amplify well for *Hieracium lepidulum.* The nine primers were screened over ten samples of *H. lepidulum* and two samples of *H. pilosella* were used as a control. Microsatellites were amplified separately in 15 µl of PCR reaction mixture containing: 1.5 µl of 10x NH_4 reaction buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl (pH 8.8), 0.1 % Tween-20), 10.68 µl of PCR-grade water, 0.6 µl of 50 mM $MgCl_2$, 0.6 µl of 2 mM dNTP, 0.5 µl of 10 µM forward primer, 0.5 µl of 10 µM reverse primer, 0.12 µl of *Taq* DNA polymerase, BIOTAQ™, and 0.5 µl of genomic DNA sample (all PCR reagents supplied by BIOLINE). PCR amplification was carried out in an Eppendorf® Mastercycler EP. PCR amplification conditions were taken from Zini and Komjanc (2007). Initial denaturation was for 5 minutes at 95 °C, followed by 15 cycles at 95 °C for 30 seconds, 63.5–56 °C (-0.5 °C every cycle) and 72 °C for 1 minute, and by 22 cycles at 95 °C for 30 seconds, 56 °C for 1 minute and 72 °C for 1 minute, and a final extension of 10 minutes at 72 °C. PCR products were separated, along with a size-standard ladder (EasyLadder I, BIOLINE), on a 1.4 % agarose gel stained with ethidium bromide in 1x TAE buffer. Gels were visualised under ultraviolet light to determine if the amplification was successful.

All nine microsatellite markers produced clear PCR amplification products in the expected allele size range in the *Hieracium pilosella* control samples; this verified that the primers were all amplifying correctly. Eight of the nine microsatellite markers produced clear amplification products in the *Hieracium lepidulum* samples screened; locus HP-34 did not amplify and was therefore discarded. The eight microsatellite markers, which did produce

clear amplification products, were ordered with fluorescent dye labels attached to the 5' end of the forward primers (Table 4.2).

Locus name	Repeat motif	Primer sequence (5'–3')	Ta	Primer source
HP-10	$(CAA)_7$	(6-FAM) CGGACAGGAATTACCGAGAG GCACATGAGCTTCATTTCCTT	63	SIGMA
HP-26	$(GTT)_{14}$	(VIC) TGGGTCGATTATTGGGATTG GCACACTTGTTCCCACCAA	64	Applied Biosystems
HP-42	$(CAA)_9$	(NED) ACCGAGCCGGGTCACTA TTCCCATGAGAACTGCTGAA	60	Applied Biosystems
HP-12	$(GTT)_{10}$	(PET) CCCCTGGAGATGTGAGTTGT TTCCATTCCACCAAGGAGAC	64	Applied Biosystems
HP-9	$(GA)_{22}(GT)_{11}$	(VIC) TCTCTTCCTTCCATTCTCATTTG TCACGTCATGCTCCAATCTC	63	Applied Biosystems
HP-3B	$(GT)_{16}$	(6-FAM) CCCCAAAACTCCCAATACAT TCATTGGGACTTCCACAAGTT	62	SIGMA
HP-26B	$(GA)_{20}$	(NED) TCCATGAGACGATGTTGGAA TCACACACACACACACACACA	64	Applied Biosystems
HP-87	$(GA)_{25}$	(PET) CAGTTTCAGTCGAGTTTCATACG TCGTTCCACTGTTTGAGTCG	63	Applied Biosystems

Table 4.2: Details of the eight microsatellite loci which were amplified in *Hieracium lepidulum*. Ta – annealing temperature.

4.3.6 Microsatellite amplification

Microsatellite loci were amplified separately in all 144 individuals, using the PCR conditions outlined above. For each individual, the PCR amplification products from the eight microsatellite loci could be mixed together in two sets, comprising four different coloured fluorescent dyes, for electrophoresis. The first set consisted of locus HP-10 (6-FAM), HP-26 (VIC), HP-42 (NED), and HP-12 (PET); the second set contained locus HP-9 (VIC), HP-3B (6-FAM), HP-26B (NED), and HP-87 (PET). PCR amplification products were then mixed with Hi-Di formamide and LIZ 500 size standard (Applied Biosystems), and denatured at

95 °C for 5 minutes. The fluorescent PCR amplification products were electrophoresed on an ABI Prism® Genetic Analyzer automated sequencer (Applied Biosystems), and GeneMarker® genotyping software (version 1.71, SoftGenetics LLC®) was used for allele size scoring.

4.4 Results

Four of the eight microsatellite loci did not amplify at a sufficient resolution in *Hieracium lepidulum* to be used for genetic analysis; these unsuccessful loci were HP-26, HP-12, HP-26B, and HP-87. The remaining four microsatellite loci (HP-10, HP-42, HP-9, and HP-3B) were examined for polymorphism within and among populations.

The four microsatellite loci which amplified appropriately in the 144 *Hieracium lepidulum* individuals were found to all be monomorphic; there was no variation present among individuals in the alleles detected. Each of the 144 *Hieracium lepidulum* individuals which were analysed had identical microsatellite 'fingerprints' (genotypes). Only one or two alleles were amplified at each microsatellite locus; homozygosity and heterozygosity at the four microsatellite loci were clearly differentiated. All individuals were homozygous at Locus HP-10, each possessing only one allele of 158 base pairs (Figure 4.1). Locus HP-42 was heterozygous in all individuals, with the two alleles 135 and 147 base pairs in size (Figure 4.1). Loci HP-9 and HP-3B were also heterozygous; two alleles per locus were amplified in each individual, with sizes of 88 and 95 base pairs (HP-9), and 121 and 131 base pairs (HP-3B) (Figure 4.2). The genetic homogeneity among individuals in these microsatellite loci prevents any separation of individuals into specific populations, locations or clonal lineages.

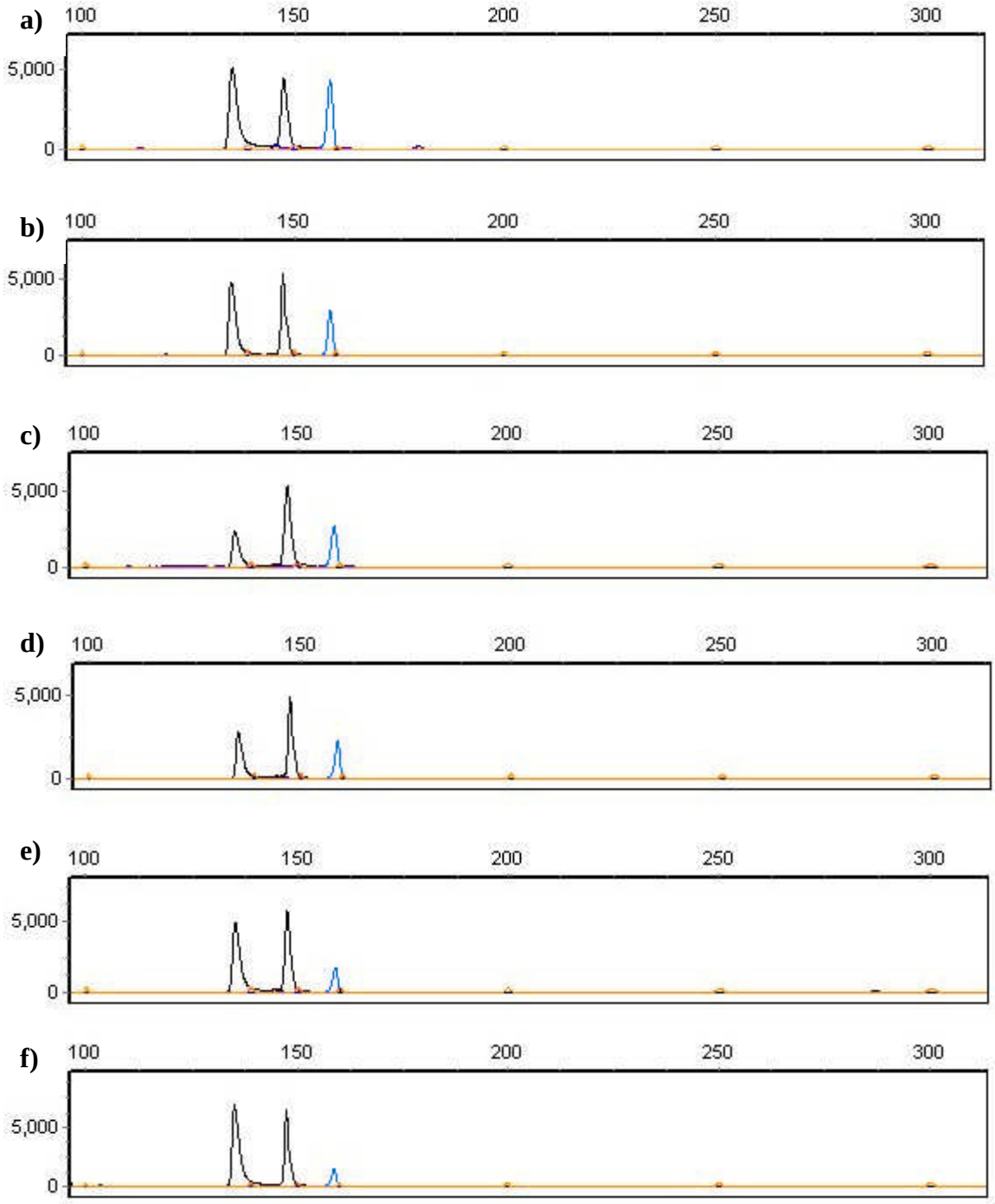


Figure 4.1: Electropherograms of locus HP-10 (blue) and HP-42 (black) illustrating the lack of microsatellite variation among a selection of individuals from; (a) Broken River lowland, (b) Broken River forest, (c) Broken River alpine, (d) Cheeseman lowland, (e) Cheeseman forest, and (f) Cheeseman alpine. Locus HP-10 is homozygous, shown by one peak of 158 base pairs in size. Locus HP-42 is heterozygous, two peaks of 135 and 147 base pairs in size. The horizontal scale denotes fragment size (base pairs) and the vertical scale is the signal intensity (Relative Fluorescent Units, RFUs).

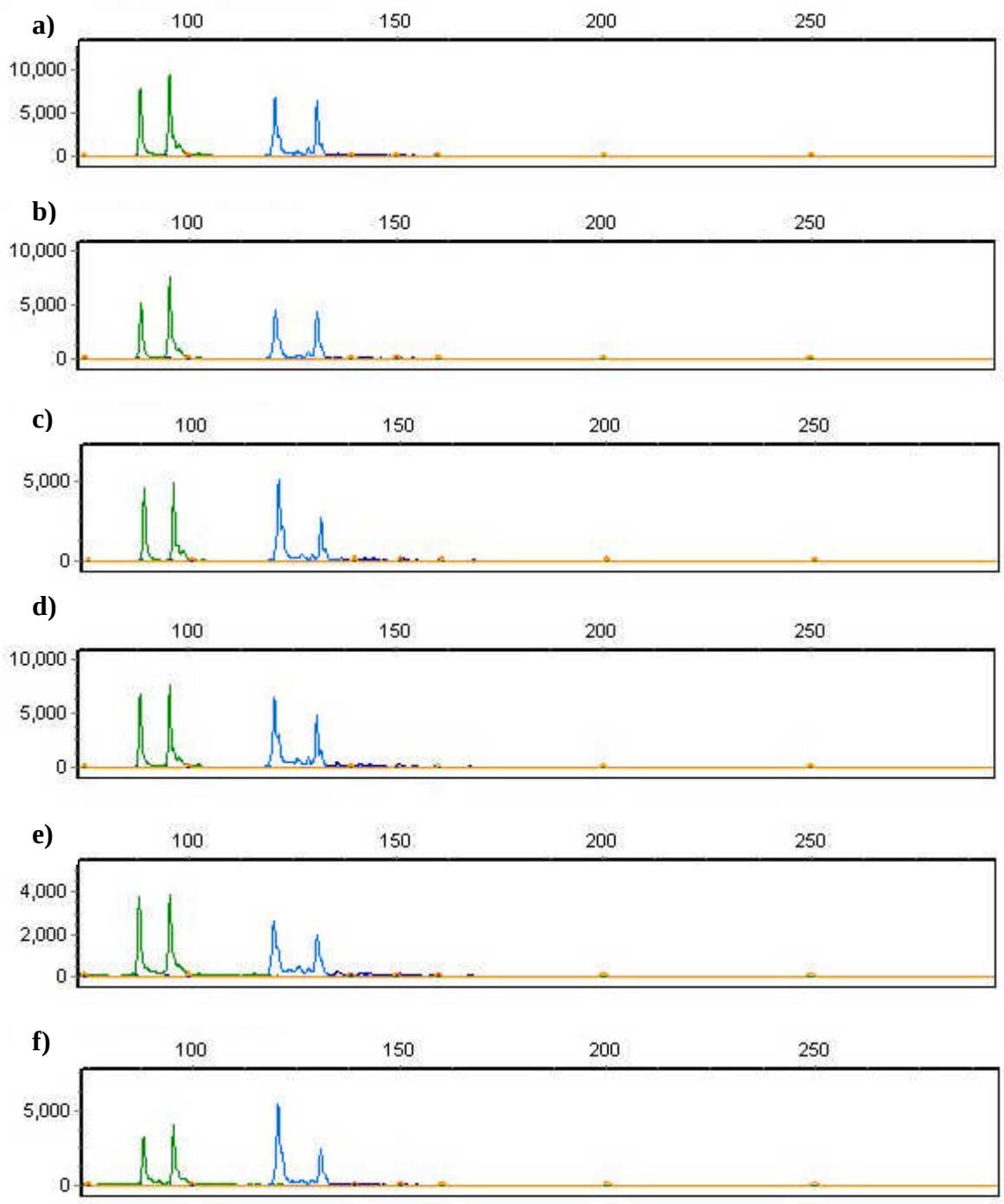


Figure 4.2: Electropherograms of locus HP-9 (green) and HP-3B (blue) illustrating the lack of microsatellite variation among a selection of individuals from; (a) Cheeseman lowland, (b) Cheeseman forest, (c) Cheeseman alpine, (d) Craigieburn lowland, (e) Craigieburn forest, and (f) Craigieburn alpine. Both loci are heterozygous. Locus HP-9 has two peaks of 88 and 95 base pairs in size, and locus HP-3B has two peaks of 121 and 131 base pairs in size. The horizontal scale denotes fragment size (base pairs) and the vertical scale is the signal intensity (Relative Fluorescent Units, RFUs).

4.5 Discussion

Nine highly variable microsatellite marker loci were developed in *Hieracium pilosella* (Zini and Komjanc 2007), of which only four amplified to a sufficient resolution in *Hieracium lepidulum*. This level of amplification across-species within a genus is similar to that found in other studies (Jump *et al*. 2002, Andrew *et al*. 2003, Hale *et al*. 2005, Lemes *et al*. 2007, Panova *et al*. 2008, Xu *et al*. 2008). These four microsatellite loci (HP-10, HP-42, HP-9 and HP-3B) were amplified in 144 individuals and were found to be monomorphic; there was no variation present among individuals in the alleles detected. This suggests that the *Hieracium lepidulum* individuals, sampled from three different habitats within three geographically-separated locations, all belong to the same clonal lineage.

The genetic homogeneity among individuals in these microsatellite loci can be explained by the breeding system of this species. *Hieracium lepidulum* is a triploid, diplosporous, obligate apomict; there has been no evidence of sexual reproduction occurring in this species (Chapman *et al*. 2004). This asexual reproduction through seed lacks meiosis, fertilisation and recombination; it is essentially a complete transmission of the entire maternal genotype to the offspring (Asker and Jerling 1992, Kultunow *et al*. 1995, Chapman *et al*. 2004). The genomes of obligate apomicts are inherited as large linkage groups, where mutations are the only source of variation between generations (Paun and Horandl 2006). Clonal lineages could also experience serious reductions in allelic variation at microsatellite loci when founding new populations with a single colonist (Paun and Horandl 2006). Due to a complete lack of variation in the four microsatellite loci amplified, there was no way to separate individuals into specific populations, locations or clonal lineages; supporting the theory that

populations all belong to the same lineage and were probably founded by a small colonising population. The genetic homogeneity among the individuals could be due to the fact that clonal organisms are expected to exhibit low evolutionary responsiveness and take a longer time to recover genetic variation after a founder event (Chapman *et al*. 2000, Paun and Horandl 2006).

Three of the four microsatellite markers amplified in the 144 *H. lepidulum* individuals exhibited fixed heterozygosity. Apomictic reproduction and polyploidy commonly result in high levels of heterozygosity or fixed heterozygosity (Bierzychudek 1989, Andrew *et al*. 2003, Comai 2005, Andersen *et al*. 2006, Paun and Horandl 2006). Fixed heterozygosity is advantageous for a species; it is thought to buffer the deleterious effect of mutations and prevents inbreeding depression (Comai 2005). High levels of heterozygosity in polyploids is also associated with increased vigour and fitness, and the success of polyploids by being able to occupy broader ecological niches (Asker and Jerling 1992, Soltis and Soltis 2000, Joly and Bruneau 2004, Comai 2005, Andersen *et al*. 2006). This fixed heterozygosity is believed to cause a heterosis effect; the success of polyploids is usually greater than that of corresponding sexual diploids (Bierzychudek 1989, Ellstrand and Schierenbeck 2000, Soltis and Soltis 2000, Comai 2005). If fixed heterozygosity leads to increased vigour and performance in *H. lepidulum*, it confers an advantage of reproducing by apomixis; these favourable genotypes will not be lost due to recombination as may be the case in sexual reproduction. This could be the reason why sexual diploids have not been discovered in *H. lepidulum*.

These results, in relation to the invasion success of *H. lepidulum,* reveal the reasons for this species' great abundance in the Craigieburn Range. Obligate apomicts are usually associated with evolutionary dead ends, as clonal lineages harbour very low levels of genetic variation, mutation being the only source. *H. lepidulum* is triploid and exhibits fixed heterozygosity which is associated with increased vigour and fitness, and the ability to invade a wide environmental gradient (Soltis and Soltis 2000, Joly and Bruneau 2004, Comai 2005, Andersen *et al*. 2006); this circumvents the decrease in fitness thought to be connected with purely apomictic reproduction. The significance of these findings in relation to earlier studies (Chapman *et al.* 2004, pers. obs. 2005), and the management and biocontrol of this weed will be covered in the general discussion (Chapter 5).

4.6 Summary

Microsatellites are the preferred molecular markers for studying polyploid, clonal organisms for several reasons, most importantly, they have high resolution, high mutation rates, are codominant and highly variable (Parker *et al.* 1998, Vasut *et al*. 2004). Clonal organisms which do not undergo meiosis, fertilisation or recombination due to the lack of sexual reproduction, only accumulate genetic variation via mutation (Kultunow *et al*. 1995, Chapman *et al*. 2004, Paun and Horandl 2006). As microsatellites have a high mutation rate, in the order of 10^{-5}–10^{-2} (Jarne and Lagoda 1996, Procaccini and Mazzella 1998), they are the best molecular markers to use in clonal organisms, such as *Hieracium lepidulum,* for discovering genetic variation, which the only source is mutation. Codominant markers are essential in population studies of polyploids, to enable allelic-size scoring for all the alleles present; dominant markers can not distinguish among alleles at a single locus (Sunnucks

2000, Jasieniuk and Maxwell 2001, Vasut *et al*. 2004). *H. lepidulum* is triploid and can potentially have three alleles amplified per locus, so codominant microsatellite markers are therefore necessary for population genetic analysis. The four microsatellite marker loci which were amplified in the 144 *H. lepidulum* individuals in this study were monomorphic; each individual possessed identical microsatellite genotypes. These results suggest that these populations from the Craigieburn Range:

1) Reproduce solely by apomixis.

2) Belong to the same clonal lineage.

3) Individuals do not harbour significant levels of mutation as they were not resolved by the highly mutatable microsatellite markers.

4) The three geographically-separated catchment locations, from ~750 m – 1450 m in altitude which represents an environmental gradient, were colonised with a single genotype which has spread by purely asexual means.

5) The fixed heterozygosity and polyploid nature of this species is probably responsible for its invasion success.

Chapter 5

5. General discussion and conclusion

5.1 What is more important in the invasive success of *Hieracium lepidulum*; adaptation via natural selection acting on genetic variation, or phenotypic plasticity?

Populations of *Hieracium lepidulum* occupying different habitats in the Craigieburn Range show distinct phenotypic variation (Miller 2005). A common garden experiment (Chapter 2) was undertaken to determine whether the phenotypic variation seen in the field is a result of phenotypic plasticity or selection and adaptation. For the common garden experiment populations were sampled from three replicate altitudes within three geographically-separate locations in the Craigieburn Range. To determine which mechanism (phenotypic plasticity or genetic variation via selection and adaptation) is more important in the invasive success of *Hieracium lepidulum*, the plant performance of each individual was recorded over time. The plant performance measures monitored were:

- maximum leaf length
- number of flowering stems
- number of buds
- number of inflorescences
- number of days until first flower
- number of seed heads
- number of seeds per seed head

These phenotypic traits exhibit distinct variation among the different habitats occupied by *H. lepidulum* in the field (Miller 2005). If *H. lepidulum* individuals from different environments loose their morphological/physiological differences in a common garden, then this species is plastic. However, if the phenotypic differences are maintained in a common garden, these differences must be genetic (via natural selection and adaptation).

Plant performance traits showed no significant statistical differences:

1) Among habitats. This suggests there is no genetic variation for these performance traits among habitats; consequently excluding the mechanism of selection and adaptation, as selection requires genetic variation on which to act. These results strongly suggest that *H. lepidulum* individuals from the Craigieburn Range reproduce solely by apomixis, and confirm the mechanism of phenotypic plasticity.

2) Among locations. This implies there is no genetic variability for these performance measures among the three locations, suggesting that the populations originated from the same source population, with no dispersal barriers.

3) Among populations. This indicates there is no genetic variation among all nine populations for the performance traits measured here; it is likely populations belong to the same clonal lineage.

The common garden results support phenotypic plasticity, the production of multiple phenotypes from a single genotype in response to environmental conditions, as the mechanism involved in the invasive success of *H. lepidulum*. Phenotypic plasticity is thought to be important in the process of invasion as it provides the colonising species with the ability

to succeed in a variety of habitats and environments (Sakai *et al.* 2001, Bossdorf *et al*. 2005). Phenotypic plasticity would bestow a fitness advantage on an invading species which suffered from a lack of genetic variation, and thus preventing adaptation via natural selection (Bossdorf *et al*. 2005).

5.2 Phenotypic plasticity in response to environmental stress

The objective of Chapter 3 was to investigate the genetic variation, phenotypic plasticity and genetic variability for plasticity in populations of *Hieracium lepidulum* subjected to two types of abiotic 'stress', shade and drought (compared to an 'optimal' control). Studies on growth under 'stressed' conditions can help in the understanding of functional trade-offs, selection pressures and evolutionary trends that may be obscured under optimal conditions (Pigliucci *et al*. 1995, Volis *et al.* 2002). The populations in this study were sampled from three different habitats, representing three altitudes, within the Broken River catchment. The same phenotypic traits measured in the common garden were used in this experiment.

For the seven performance traits studied, only four of the reproductive traits showed significant plasticity in response to the 'stress' treatments. The phenotypes produced in the shade treatment were very distinct compared to those in the drought 'stress' and the control. Populations exhibited low plasticity in response to the drought treatment compared to the control for all phenotypic traits studied; this suggests that *Hieracium lepidulum* populations are drought tolerant. The three populations displayed significant plasticity in response to shade 'stress'. The shade treatment induced a significant reduction in the number of buds, flowers and seed heads produced and a considerable delay in flowering time. The results

suggest that *H. lepidulum* is shade intolerant, which is evident in its scarcity in nature in closed-canopy forest habitats.

The possibility of *H. lepidulum* being drought tolerant is not advantageous from the standpoint of preventing this species' invasive spread. Drought tolerance confers a significant fitness advantage as individuals would not only have the ability to successfully invade novel, arid environments, but would also be able to thrive in existing habitats when faced with a drought. Also, as the reproductive traits were not affected by drought 'stress' it potentially could allow populations to spread into areas occupied by plants which cannot tolerate drought conditions. On the other hand, *H. lepidulum* appears to lack the ability to prosper in light-limiting conditions. Invasion into shaded habitats seems to be restricted due to the reduced reproductive output under these conditions; this is especially pertinent in the Craigieburn Range due to the great expanse of native *Nothofagus* forest.

5.3 Genetic diversity and population genetic structure

Understanding of genetic diversity and population genetic structure is necessary for evolutionary studies of mating systems and relatedness; it is also a requirement for successful weed management programmes (Jasieniuk and Maxwell 2001, Sun *et al*. 2001, Hufbauer 2004). Population genetics can help us comprehend the biology of invasions, which will aid in weed control by revealing which species are present, the invasion origin and the methods and distance of migration (Jasieniuk and Maxwell 2001, Walker *et al*. 2003, Hufbauer 2004).

Microsatellites are the preferred molecular markers for studying polyploid, clonal organisms for several reasons, most importantly, they have high resolution, high mutation rates, are codominant and highly variable (Parker *et al*. 1998, Vasut *et al*. 2004). Clonal organisms which do not undergo meiosis, fertilisation or recombination due to the lack of sexual reproduction, only accumulate genetic variation via mutation (Kultunow *et al*. 1995, Chapman *et al*. 2004, Paun and Horandl 2006). As microsatellites have a high mutation rate, in the order of 10^{-5}–10^{-2} (Jarne and Lagoda 1996, Procaccini and Mazzella 1998), they are the best molecular markers to use in clonal organisms, such as *Hieracium lepidulum*, for discovering genetic variation, which the only source is mutation. Codominant markers are essential in population studies of polyploids, to enable allelic-size scoring for all the alleles present; dominant markers can not distinguish among alleles at a single locus (Sunnucks 2000, Jasieniuk and Maxwell 2001, Vasut *et al*. 2004). *H. lepidulum* is triploid and can potentially have three alleles amplified per locus, so codominant microsatellite markers are therefore necessary for population genetic analysis.

The four microsatellite marker loci which were amplified in the 144 *H. lepidulum* individuals in this study found that the loci were monomorphic; each individual possessed identical microsatellite genotypes. These results suggest that these populations from the Craigieburn Range:

1) Reproduce solely by apomixis.

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3) Individuals do not harbour significant levels of mutation as they were not resolved by the highly mutatable microsatellite markers.

4) The three geographically-separated catchment locations, from ~750 m–1450 m in altitude which represents an environmental gradient, were colonised with a single genotype which has spread by purely asexual means.

5) The fixed heterozygosity and polyploid nature of this species is probably responsible for its invasion success.

Previous work on New Zealand populations of *H. lepidulum*, using intersimple sequence repeats (ISSRs) (Chapman *et al.* 2004) and allozymes (pers. obs. final-year BSc project 2005), have found there to be low levels of genetic variation within populations, and high levels of variation among populations. Five populations were used in both studies from three geographically-separated areas in the South Island; Canterbury, Kaikoura and Otago. Details of the population sites are presented in Table 5.1. Multivariate cluster analysis and principal coordinate analysis clearly differentiated the Canterbury, Kaikoura and Otago populations, showing greater variance among populations than within (Chapman *et al.* 2004).

Site	**Altitude (m)**	**Age and density of population**	**Habitat**
Rob Roy, Otago	1530	Invading front; plants sparsely scattered	*Chinochloa, Celmisia*, below glacier
Pisa, Otago	1700	Invading front; common in sheltered areas, sparsely scattered on windswept plateau	Plateau of Pisa Range, sheltered from the wind in the escarpment lip
Lochar Burn, Otago	390	At least 15 years old; dense mat	Oversown pasture/*H. lepidulum* grassland
Broken River, Canterbury	500–1300	Recorded in the vicinity as 'rare' 1962; common	Confined to near the edge of *Nothofagus* forest
Mt Fyffe, Kaikoura	1250–1440	At least 20 years old; scattered clumps	Roadside and subalpine grassland

Table 5.1: Site details of the five *Hieracium lepidulum* populations used in previous studies (Chapman *et al.* 2004, pers. obs. final-year BSc project 2005); modified table from Chapman *et al.* (2004).

The differences in the levels of genetic variation detected between the previous studies (Chapman *et al.* 2004, pers. obs. final-year BSc project 2005) and this microsatellite analysis (no genotype variation) can be explained in several ways:

1) The scale of sampling: The geographic separation of the populations in this study is only ~10 km, whereas the geographic separation in the other studies is huge in comparison, in the order of ~300–500 km. It is therefore possible that the Otago and Kaikoura populations came from different seed sources when they were first introduced.

2) Populations from the Craigieburn Range are the oldest in New Zealand, recorded in 1941 (herbarium records), and were likely founded by a source comprising of a single clonal lineage.

3) Fewer loci (only four) amplified to a sufficient resolution with the microsatellite markers. With six ISSR primers which screen the whole genome, considerably more loci are compared. The allozyme results included six enzyme systems, and may have shown genuine variation although with allozymes, the possibility of selection can not be excluded. The greater number of loci which are able to be screened will increase the sensitivity of the analysis.

4) The microsatellite primers were designed for *Hieracium pilosella*. Amplifying across-taxa, even among closely-related species, typically result in a decrease in microsatellite variation (Hale pers. comm.).

Together the studies have certainly demonstrated low levels of variation within populations of *H. lepidulum*; the residual variation probably resulting from somatic mutations. The previous studies (Chapman *et al.* 2004, pers. obs. final-year BSc project 2005) which found

genetic variation among geographically-separated populations suggests that the Otago, Canterbury and Kaikoura populations were all founded by different sources; each population could therefore harbour different clonal lineages.

5.4 Summary and the potential for control

5.4.1 Summary

Populations of *Hieracium lepidulum* were sampled from three distinct altitudes which represented different habitats, within three geographically-separate locations in the Craigieburn Range. Population genetic analysis found that there was no genetic variation among any of the individuals, strongly suggesting that in the Craigieburn Range this species reproduces purely by apomixis. Individuals all possessed identical microsatellite genotypes, which implies that they belong to the same clonal lineage. *H. lepidulum* appears to have overcome the reduction in fitness associated with apomictic reproduction by phenotypic plasticity, fixed heterozygosity and polyploidy, which are all associated with increased vigour, fitness, and the ability to occupy broader ecological niches (Bierzychudek 1989, Asker and Jerling 1992, Soltis and Soltis 2000, Joly and Bruneau 2004, Comai 2005, Andersen *et al*. 2006). Apomixis results in a colossal number of asexual clones, enabling the rapid proliferation and invasive spread of *H. lepidulum*.

5.4.2 The potential for control

There are three major prospects for managing *Hieracium lepidulum* invasion in New Zealand; herbicides, grazing management and biological control (Espie 1994).

Herbicides and grazing management:

The ability to control *H. lepidulum* with herbicides or grazing management is not promising due to its triploid nature, fixed heterozygosity and phenotypic plasticity. These traits are all associated with increased fitness, vigour, and conferring greater tolerance (Bierzychudek 1989, Asker and Jerling 1992, Soltis and Soltis 2000, DeWalt and Hamrick 2004, Joly and Bruneau 2004, Comai 2005, Andersen *et al*. 2006). Polyploidy can bestow an advantage through gene redundancy; all genes have a duplicated copy that is available for evolutionary experimentation (Comai 2005). The ability to diversify gene function by altering redundant copies of important or essential genes (Comai 2005), may lead to development of resistance against herbicides and grazing (possibly by the production of defence toxins). It has been reported that *Hieracium* species are tolerant to herbicides and incur high application costs (Espie 1994). Phenotypic plasticity could also prevent management through grazing from succeeding. Plastic genotypes confer the ability to alter morphology, physiology, behaviour, life history, growth, and demography (Miner *et al*. 2005). Grazing management could induce a plastic response in *H. lepidulum*; transforming to flat, mat-forming growth and delaying reproduction could avoid the detrimental effect of grazing. Grazing consistently increased the cover of the prostrate mat-forming *Hieracium pilosella* (Espie 1994); this is potentially what may occur if *H. lepidulum*, through phenotypic plasticity, alters its growth and reproduction in response to grazing pressures. Application of herbicides and grazing management is not

suitable for conservation land; it may also have a negative impact on the protected indigenous flora.

Biological control:

The highly-variable microsatellite marker loci detected a complete lack of variation; individuals all comprised the same microsatellite genotype. This result indicates that genotype-specific pathogens may potentially be effective as biocontrol agents against this invasive weed. However, no identification of host-specific pathogens has been made yet. Host-specific fungi have been introduced to control another apomictic species, *Hieracium pilosella*, based on a similar argument; however it has not been convincingly successful (Chapman pers. comm.).

Biological control using insect enemies of *Hieracium* could potentially have the most success. In New Zealand *Hieracium* species are attacked by a wide range of native and exotic phytophagous insects (Syrett and Smith 1998). However, insect damage is rarely observed and none of the insect species which specialise on *Hieracium* species in Europe have been found in New Zealand (Syrett and Smith 1998). There are specialist gall-forming insects which cause considerable damage to *Hieracium* species in Europe; introducing an insect biological control agent like this could potentially manage the *Hieracium* invasion (Syrett and Smith 1998).

Recent studies (Grosskopf *et al.* 2002) have investigated the potential of two insect species, *Cheilosia urbana* and *Cheilosia psilophthalma*, as candidates for biological control agents of

Hieracium in New Zealand. Both hoverfly species specialise on *Hieracium* in Central Europe, and physiological host-range tests confirmed that they were genus-specific; this is promising for their safe release in New Zealand as there are no native *Hieracium* species to be concerned about (Syrett and Smith 1998). Females lay eggs on the leaf axils; *C. urbana* larvae move into the soil and feed externally on roots resulting in small holes, whereas *C. psilophthalma* larvae bore into leaf axils, rosette centres, the basal parts of stolons and stolon tips (Syrett and Smith 1998). The reproductive biology of *Hieracium lepidulum* differs from the other three weedy species of *Hieracium* in New Zealand, as it does not produce stolons (Espie 1994, Syrett and Smith 1998). This suggests that *C. urbana* may be a more effective biological control agent for *Hieracium lepidulum* as it damages the roots; however, *C. psilophthalma* may inflict enough damage to leaf axils and rosette centres to also aid in control.

5.5 Future work

An interesting area of future work could involve a genetic survey of populations sampled from locations all around New Zealand using a combination of molecular markers; including ISSRs and microsatellites, designed specifically for *H. lepidulum*, with more loci sampled than in this or previous studies. This would aid in the understanding of the evolutionary colonisation processes involved in the invasive spread of *Hieracium lepidulum*. Understanding the population genetic structure of *H. lepidulum* in New Zealand is crucial for predicting rates of spread and the likelihood of the evolution of resistance to biocontrol agents.

From the standpoint of the biological control of this invasive weed, further research is needed to determine the safety of introducing an insect biological control agent as a candidate for managing the *Hieracium* invasion; it is not productive to introduce a biocontrol agent which will then cause damage to indigenous species. As *H. lepidulum* does not spread by stolons (Espie 1994, Syrett and Smith 1998), a seed predator may potentially be the most damaging biological control agent for this species; this warrants further research.

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